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THE YALE JOURNAL
OF BIOLOGY AND MEDICINE

Founded in 1928.

George H. Smith; *Editor-in-Chief, 1928-49; Editor-in-Chief Emeritus, 1949-52.*

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Subscriptions. THE YALE JOURNAL OF BIOLOGY AND MEDICINE is published in September, November, December, February, April, and June from 333 Cedar Street, New Haven 11, Connecticut, U.S.A. The subscription price for one volume of six issues is $7.50 in the United States and other countries. For students registered in any school of Yale University, the subscription price is $4.00 per volume. Single copies are $1.50 each except for special issues.

Entered as second-class matter January 11, 1929, at the post office at New Haven, Connecticut, under the Act of March 3, 1879.

PRINTED BY THE VAN DYCK PRINTING CO., NEW HAVEN, CONN.

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ENDOCRINOLOGICAL STUDIES ON THE EURYHALINE KILLIFISH, *FUNDULUS HETEROCLITUS (Linn.)***

The common killifish of the eastern United States, *Fundulus heteroclitus* (Linn.), is widely employed as an experimental animal by zoologists, embryologists, and comparative physiologists. The late Dr. Myron Gordon[82] points out that it is a hardy species of convenient size, readily caught in tidal pools but not usually bred in aquaria for purposes of raising the stock. Dr. Gordon warns against confusion with the striped killifish, *Fundulus majalis,* which is more delicate and will not stand up as well under aquarium conditions.

During the winter, in the latitude of New Haven, Connecticut, *F.heteroclitus* passes into a state of sexual regression, hibernating in the mud. In spring it may be caught in large numbers in estuarine waters where it breeds in the reed beds during June and July. The young become sexually mature the following year, at a length of about one inch, and fish of approximately 3 inches in length are usually in their second year. Unlike *F.majalis, F.heteroclitus* is completely euryhaline, moving freely from salt to fresh water. It may be kept indefinitely in either medium under laboratory conditions and is tolerant of standing water provided the tanks are well aerated and not overcrowded.

Endocrinological studies on *Fundulus heteroclitus* were begun in the Bingham Oceanographic Laboratory in 1947. At that time a number of investigations, reviewed by Atz,[62] had been concerned with the hormonal regulation of the reproductive cycle, Albert[1] had studied the thyroid and exophthalmos, and a substantial body of work, reviewed by Parker,[53] had been devoted to the chromatophore-regulating hormones. Dr. Ernest F. Thompson and I were interested in a different problem: the "year marks" on the scales of fishes. The formation of the annulus is correlated with the onset of spring growth, and we thought that it might be possible to induce an artificial off-season annulus by administration of growth hormone. Although our first experiment was, in this respect, unsuccessful it served to

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** Supported by grants from the National Science Foundation: G-50 (1952-1954), G-941 (1954-1957), G-4001 (1957-1960).

Received for publication May 4, 1959.

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show that purified beef growth hormone enhances the growth rate of fish.[60] We had much to learn about procedures in handling the fish to minimize collateral shock effects and dietary disturbances.[57, 58] We now know that injections should only be given on alternate days, the fish should be fed in the morning and injected later in the day, and they should be handled under anesthesia with tricaine methane sulfonate. A little iodine must be added to the Aronson's mixture used as food to prevent development of goiter, and the diet must be supplemented with fresh frozen brine shrimp or daphnia to prevent disturbances attributed to cumulative vitamin deficiency.*

The first experiments were made on normal fish, but it was quickly realized that hypophysectomy is necessary for decisive results. The operation is an easy one and fish rarely die as a direct result of surgery.[57] After removal of the pituitary, growth in length ceases although weight changes are erratic, the gonads and thyroids pass into regression but the interrenal (adrenocortical) tissue shows little change. The fish become anemic, there is a gradual loss of melanin pigment from the skin, a disturbance of calcium metabolism is reflected in the frequent occurrence of kidney stones, and the fish are unable to survive in fresh water. Each of these endocrine disturbances has been subjected to further analysis, reviewed below, but there remain many unresolved problems.

GROWTH HORMONE

A renewal of growth can be elicited in hypophysectomized Fundulus by chronic administration of purified beef growth hormone (GH).[58] The treatment also induces new growth at the margin of the scales, with the formation of an annulus resembling the natural "year mark." For effective results a dose of 10 μg. per gram weight, administered thrice weekly for at least 5 weeks, is necessary and longer periods are desirable. In comparison with hypophysectomized rats, in which the effective dose is equivalent to 0.1 μg. per gram weight daily for 10 days, two distinct problems were evident: the slowness of onset of the response and the fifty-fold greater dose requirement. Various aspects of these problems have been subjected to experimental investigation.

In the first place, it was thought that Fundulus might be more responsive to growth hormone derived from fish. Dr. Alfred E. Wilhelmi, who pro-

* This problem has not been investigated in Fundulus, although there is an extensive literature on the subject in respect to hatchery diets for trout. However, we observed that when Fundulus were kept for many months on a cooked liver-pablum diet, they not only developed goiter, but also showed a disturbance of the nervous system (paroxysms of spinning) and severe ceroid disease of the liver.

vided the purified beef growth hormone, became interested in the problem and offered to attempt the preparation of a purified fish growth hormone, provided we could supply a sufficient quantity of frozen glands. This was a major project. As the result of three expeditions, in the summers of 1950, 1952, and 1954, working on freshly captured fish at Jackson's wharf on Campobello Island, N. B., we collected totals of *ca.* 350, 400, and 950 grams respectively, representing an estimated 114,700 heads of pollack, hake, and cod. The most successful fish growth hormone preparation proved to have a molecular weight that was approximately one-half that of beef growth hormone.[75]. It was 55 per cent as effective as beef growth hormone when administered to hypophysectomized killifish at an equivalent dose in μg. per gram weight.[59] However, unlike the beef growth hormone, the fish growth hormone was free from thyroid-stimulating activity. When given in conjunction with mammalian TSH, the growth-promoting activity of the fish preparation was enhanced.[62] Even under these circumstances, however, there was no evidence that fish growth hormone, derived either from pollack or hake, can elicit a greater growth response than beef growth hormone.

Antibody formation is another factor that might limit the response to heterologous growth hormone preparations. We have made no direct study of this problem, but are inclined to minimize its importance. During an 8-week experiment there was continuous slow growth, either with fish or beef growth hormone, and no evidence suggestive of a decreasing effectiveness after prolonged treatment.

These considerations lead, however, to a discussion of the specificity of growth hormones derived from different sources.[29] Although Fundulus is stimulated by beef growth hormone, the rat is totally unresponsive to fish growth hormone.[62, 78] During the past year we have found that Fundulus responds poorly to human, monkey, and pig growth hormone, but sheep growth hormone is as effective as beef growth hormone.[61, 64] There may be similar differences between growth hormones derived from different species of fish.

Temperature is another factor that might account for the greater response of warm-blooded animals, as compared with fish, to beef growth hormone. In one experiment, beef growth hormone was administered to hypophysectomized Fundulus maintained at 10°, 15°, 20°, and 25° C.[62] The growth response was negligible at the lowest temperature, very slight at 15° C., and maximally subequal at 20° and 25° C. In a subsequent unpublished investigation, it was found that although hypophysectomized Fundulus, like intact individuals, can live at higher temperatures

(ca. 30° C.) they cannot then tolerate the trauma of handling and injection. The results of these experiments suggest that a temperature of 20-25° C. is optimal for the growth response and that higher temperatures are inhibitory.

The condition of the fish is an important factor in regulating the onset and magnitude of the growth response. In earlier experiments, in which conditions were not optimal, there was often a lag of 2-3 weeks before any significant growth increment became evident, and this was frequently accompanied by an initial weight loss. Under the improved procedures now employed, we usually observe an onset of renewed growth at the end of the first week, although the increment may not be statistically significant so early in the experiment. The magnitude of the percentage length increase varies from one group of fish to another, even when the same dose of standard beef growth hormone (NIH 1) is employed. However, for a given group of fish, there is a linear log dose-response curve with a minimum effective dose reflected in a slight growth response at 3 μg. per gram weight.[61]

A major cause of the difference between one group of fish and another can almost certainly be attributed to the length of time that has elapsed after hypophysectomy. Experience has shown that a period of at least 2 months, at 20° C., must elapse before the target organs of the pituitary of Fundulus pass into stabilized regression. Olivereau[80] had the same experience with the eel, in which over 7 months had to pass before the thyroid became inactive. If growth hormone injections are begun one month after hypophysectomy, the killifish are more responsive than if a period of 6 months has elapsed. In any experiment with an unknown growth hormone preparation, it is necessary to have two control groups, one receiving saline injections and one a standard beef growth hormone preparation.

THYROID AND THYROTROPIN

The thyroid of Fundulus, like that of the majority of teleosts, is an unencapsulated gland with dispersed follicles in the vicinity of the ventral aorta and afferent branchial arteries.[85] Gorbman and Berg,[81] using tracer doses of I^{131}, showed that at 22° C. the uptake of radioactive iodine by the thyroid of *Fundulus heteroclitus* reached a peak of 10 per cent after 24 hours in salt water. The peak was three times greater when the fish were kept in water of low salinity. The marine species, *F.majalis,* gave similar results but there was a lesser stimulation in dilute sea water. The freshwater species, *F.diaphanus,* showed a higher uptake at 15° C. than at 8° C., and

the peak was attained very slowly, after one week. The following products of hormonal iodine synthesis were identified: monoiodotyrosine, diiodotyrosine, and thyroxine. In *F.heteroclitus* in salt water there was also an unidentified compound.

We have made no study of the controversial problem of the metabolic function of the thyroid in fishes.[37, 62] Histological examination suggests that the thyroids of male *F.heteroclitus* are more active than those of females, and that there is a liquefaction and resorption of colloid in spring.[57] The experiments of Burden[8] and Harris,[36] discussed below, tend to exclude participation of the thyroid in freshwater adaptation.

The thyroid of teleosts with diffuse glands cannot be surgically removed, but a number of investigators have attempted radiation thyroidectomy.[62] Harris[36] applied this method to Fundulus, but the difficulties were the same as those encountered by other workers in various teleostean species. A single high dose of I^{131} may destroy the functional follicles but, after a few weeks, new follicles regenerate. These may be destroyed by repeated injection but, even after a third treatment, Harris found subsequent regeneration in the majority of cases. Those individuals which showed complete or nearly complete absence of thyroid follicles were the ones that died, but the possibility of radiation injury to other organs is not excluded. The survivors were found to be more or less severely hypothyroid, both by histological criteria and by an inability to retain a tracer dose of I^{131}.

Injections of thiourea result in the expected hypertrophy and hyperplasia of the thyroid of Fundulus,[13] indicating enhanced secretion of thyrotropin (TSH) from the pituitary. This hormone is certainly present in the hypophysis since injections of Fundulus pituitary brei stimulate thyroid cell height in recipients of this species.[8]

Albert[1] found that the thyroid of Fundulus responds to injections of mammalian TSH. He observed a slight change after 6 hours, by histological criteria, followed by progressive evidence of stimulation resulting in marked hypertrophy and hyperplasia after 3 days. The dose was stated to be 200 mg. equivalents of fresh sheep pituitary.

Our studies of the thyroid have been incidental to an evaluation of contaminating traces of TSH in growth hormone preparations. It was shown, in an investigation directed to this problem,[60] that the thyroid of Fundulus is extremely sensitive to chronic treatment with small doses of TSH. A dose of the order of 0.4 mU per gram weight, administered thrice weekly for one month, stimulated the thyroid cell height of hypophysectomized recipients to somewhat more than normal levels. Even a ten times lesser dose had some effect on 2 out of 6 fish. More recently Noel Nussbaum and

I have confirmed these results by injecting a tracer dose of I^{131} at the end of the experiment (unpublished).

EXOPHTHALMOS-PRODUCING SUBSTANCE

Albert[1] showed that injections of mammalian TSH caused protrusion of the eyeballs in normal or hypophysectomized Fundulus; the response was proportional to the dose. The work of Dobyns and Steelman[16] demonstrated that an exophthalmos-producing substance (EPS), assayed on Fundulus, can be chemically separated from TSH, assayed on the chick thyroid. Both Fundulus[17, 72] and goldfish[12] have been employed for the assay of EPS in the blood serum of patients suffering from exophthalmos, and TSH is known to elicit proptosis in eels.[80] We have found that chronic treatment of hypophysectomized Fundulus with low doses of TSH (containing EPS) can maintain a slight degree of exophthalmos for the duration of the experiment.[60] On the other hand, removal of the pituitary had no effect on the degree of protrusion of the eyeballs.[62]

The experiments cited above led to the conclusion that exophthalmos is regulated by a specific pituitary hormone, separable from TSH. However, in respect to fishes, there are conflicting reports in the recent literature. Langford[48] obtained negative results with injections of TSH in *Fundulus heteroclitus* var. *bermudae,* whereas either thyroxine or triiodothyronine elicited exophthalmos. Similarly, Matty, Menzel, and Bardach[49] were unable to produce exophthalmos in the parrot fish, *Sparisoma squalidum,* with TSH. Positive results were obtained with thyroxine, 3.5.3′-triiodothyronine, and also with androgens, but not estradiol. From these, and collateral investigations on other species, the authors suggest that androgens stimulated the thyroid and that exophthalmos in fish is a direct thyroidal effect. Although the work of Dobyns and Steelman[16] showed that mammalian TSH could be separated into two fractions, one stimulating the chick thyroid and the other causing exophthalmos in Fundulus, the effect of these preparations on the thyroid of Fundulus itself was not investigated. Until such a study has been made, the problem cannot be resolved.

ADRENAL AND ADRENOCORTICOTROPIN

In the past, there was much discussion as to whether the so-called anterior inter-renal (Giacomini tissue) or the corpuscles of Stannius (posterior inter-renal) were the true homologues of the mammalian adrenal cortical tissue in teleostean fishes. The problem was resolved by Rasquin[67] who showed that in the Mexican characin, *Astyanax mexicanus,* adminis-

tration of exogenous adrenocorticotropin (ACTH) stimulated the former but not the latter target organ. A recent article by Krauter[42] may be cited in confirmation of these findings. The anterior interrenal of Fundulus is also stimulated by ACTH, although it is less responsive than that of Astyanax.[62]

Direct proof that the anterior inter-renal tissue is capable of synthesizing adrenocortical steroids has recently been obtained by Phillips and Mulrow.[55] The head kidneys of Fundulus, containing the interrenal tissue, were incubated with tritiated progesterone, and the resultant corticoids were identified in the medium by chromatographic separation of the radioactive products. The corpuscles of Stannius of Fundulus are too small for such a study, but incubation of the corpuscles of Stannius of the flounder, *Pseudopleuronectes americanus,* gave negative results by this method.[56]

Our knowledge of the circulating adrenocorticosteroids of fishes has grown rapidly in recent years. Dr. John G. Phillips,[52, 53] a Commonwealth Fellow at Yale, has surveyed a large number of species. In *Fundulus heteroclitus,* as in many teleosts, the major plasma corticosteroid is cortisol. Blood was collected from autumnal fish in winter regression and, at least at this season, the cortisol level is lower in females than in males—the opposite of what has been observed in some other species of fish. Although the small volume of plasma obtainable from many hundred specimens of the killifish did not permit detection of other corticoids that might be present in small amounts, it must be inferred from the incubation experiments of Phillips and Mulrow[55] that aldosterone is likely to occur. This mineralocorticoid has recently been demonstrated by Phillips, Holmes, and Bondy[54] to be present in the blood of the salmon, *Oncorhynchus nerka.*

The function of the adrenocortical tissue in fishes is not well understood and will not be reviewed in this article. Studies on Fundulus have contributed little to a resolution of the problem. The adrenocorticoids have no direct action in promoting survival of this species in fresh water, but they apparently play some role in hemopoiesis. These problems are discussed below, under the appropriate headings.

The anterior interrenal of teleosts also contains the adrenal medullary (chromaffin) cells. No changes can be observed in these cells after removal of the pituitary in Fundulus.[57] Functional studies are lacking in this species and but poorly investigated in other teleosts, although it is well known that exogenous adrenaline usually elicits melanophore concentration.[51] However, Breder and Rasquin[6] observed darkening in Chaetodipterus, and melanophore adrenaline insensitivity in some fishes is discussed by Rasquin.[60]

ACTH is undoubtedly present in the hypophysis of Fundulus, as in other species of teleosts.[62] The work of Rasquin[67] on Astyanax demonstrated that cold shock, or even saline injections, results in a stimulation of the anterior interrenal tissue, which may be attributed to endogenous release of ACTH. Recent studies by Slicher[74] on Fundulus blood showed that cold shock caused changes in the white cell count similar to those resulting from injections of exogenous ACTH (see under Hematology).

GONADAL HORMONES AND GONADOTROPINS

The extensive literature on the reproductive hormones of fishes has been reviewed by Hoar[37] and Atz.[62] In Fundulus, as in other species, hypophysectomy leads to atrophic changes in the gonads and regression of the secondary sexual characters.[9, 10, 46] The relative weight of the gonads (gonosomatic index) of hypophysectomized males is approximately 0.3 per cent of the body weight.[37, 62] This may be compared with a mean of 0.7 per cent during winter regression, and 5.8 per cent during the breeding season. Similar effects were observed in females. However, a period of at least 6 weeks at 20° C., and longer at lower temperatures, is necessary for the gonads to pass into complete regression. Moreover, it is undesirable to operate on the fish during the breeding season: atrophy of the mature gonads, especially in females, results in heavy mortality apparently from toxemia.

Males of *Fundulus heteroclitus* develop bright yellow bellies during the breeding season. This nuptial coloration disappears in winter and also after hypophysectomy. As might be anticipated, treatment of hypophysectomized males with androgens elicits the nuptial coloration.[10, 62] We found, in accordance with the experience of others, that methyl testosterone is a most potent androgen in fishes. Injections of methyl testosterone into castrated male Fundulus confirmed the conclusion that development of nuptial coloration is a direct response to the sex steroid. However, both earlier work of Burger[10] and more recent studies in the Bingham Laboratory[62] revealed that treatment with androgens stimulated the testis weight of hypophysectomized male Fundulus. The development of nuptial coloration, under the influence of exogenous androgen, is presumably augmented by endogenous release of the as yet unidentified natural sex steroid.

The pituitary of Fundulus is rich in gonadotropin.[47] No assays have been made at different seasons of the year, but the work of Sokol[77] has shown that gonadotropic cells of the hypophysis of this species become degranulated in winter and re-activated in spring. The nature of the gonadotropin or gonadotropins of Fundulus has not been clarified. In other

teleostean species it is claimed that both a follicle stimulating-like and a luteinizing-like gonadotropin are present.[37,61,62] The regressed testes of hypophysectomized male Fundulus are extremely sensitive to chronic treatment with minute doses of mammalian luteinizing hormone (LH).[62] As little as 0.05 μg. per gram weight of an Armour preparation, administered thrice weekly for one month, caused a statistically significant increase in the gonosomatic index. A ten times greater dose was somewhat more effective but failed to stimulate the testes to complete maturation. This might be attributed to the development of antibodies. Some evidence supporting this hypothesis was obtained indirectly, from the response to beef growth hormone containing traces of LH and TSH.[62] At 10° C. the testes were more strongly stimulated than at 15° or 20° C., a result which could be interpreted as a failure of antibody release at low temperature.[5]

Other factors may be involved, however, in the apparent failure of LH, at the doses employed, to bring about full maturation of the testes. In an experiment with TSH-free hake growth hormone and mammalian TSH, either preparation alone was found to cause some testis growth.[62] When the two preparations were given together, the effect was either additive or possibly synergic. Some individuals released flowing sperm, although the testes were subnormal in size. Similar observations have been made from time to time with mammalian growth hormone preparations containing appreciable traces of LH and TSH: a human growth hormone, for example, evoked testis growth and flowing sperm in all of the 7 recipients.[64] A synergic action of GH and (or) TSH in promoting the response to LH is strongly indicated. Further experiments are planned, but have been impeded by the difficulty of obtaining growth hormone preparations that were totally free of testis- and thyroid-stimulating properties when tested on fish.

In contrast to their great sensitivity to mammalian LH, hypophysectomized Fundulus are almost completely unaffected by mammalian follicle stimulating hormone (FSH).[62] At high doses there may be a slight stimulation of the testes, attributed to traces of LH in the FSH preparation. The problem of an FSH-like hormone in fishes cannot be resolved until purified fish gonadotropic fractions can be tested on hypophysectomized recipients.

PROLACTIN

During the course of pilot experiments with various gonadotropins, a group of hypophysectomized male Fundulus was treated with moderately well-purified prolaction.[63] No stimulation of the gonads was observed but,

to our astonishment, the fish became black. The preparation contained traces of ACTH, as shown by the stimulation of the anterior interrenal tissue, and Dr. M. Ruth Wright found frog melanophore-dispersing activity of a low order. Experiments with highly purified prolactin, intermedin, and a_s=adrenocorticotropin, made available to us through the courtesy of Dr. Choh Hao Li, were inaugurated in an attempt to clarify the problem. It was found that ACTH-free intermedin-free prolactin is capable of increasing the amount of melanin in the pigment cells. The ghosts of melanophores that had become depigmented as the result of hypophysectomy were restored to normal coloration, but no new pigment cells appeared. Highly purified intermedin (β-MSH), which had only an insignificant effect on the melanin assay, induced proliferation of newly formed melanocytes which spread over the normally unpigmented belly. The requisite dose was a high one, but low doses of intermedin that had no effect when administered alone were strongly potentiated by simultaneous treatment with prolactin. More recent studies have confirmed these results and enlarged our understanding of the problem.[40] Alkali-treated intermedin is more effective than untreated. Alpha-corticotropin, which has a slight effect similar to that of intermedin, is also potentiated by prolactin. Prolactin is evidently essential for a significant increase of melanin synthesis *in vivo* and, in the absence of this hormone, the proliferation of new melanocytes by intermedin is retarded.

However, *in vitro* studies by Bernard Kosto and Dr. Morris Foster, who collaborated in this investigation, demonstrated that chronic treatment with either prolactin, intermedin, or ACTH stimulated the ability of the tail fin tissue to oxidize the melanin precursor, dopa. The preparations darken during incubation with the substrate, and there is a correlated rise in oxygen consumption. As a tentative conclusion it would seem that in the living animal prolactin promotes an increased supply of melanin substrate, which is apparently lacking after hypophysectomy. On the other hand, a variety of hormonal treatments stimulate the "dopa oxidase" enzyme system, as can be demonstrated when exogenous substrate is provided *in vitro*.

These interesting observations led to an attempt to confirm the suspected presence of prolactin in the fish hypophysis. Earlier studies by other investigators, using the pigeon crop test, had indicated an affirmative answer.[69] Meanwhile, Grant and Grant,[33] using hypophysectomized recipients, had confirmed the findings of Chadwick that prolactin specifically elicits the "water drive" response in the terrestrial red eft stage of the newt, *Diemyctylus viridescens*. This assay method has now been applied to fish pituitary preparations, including Fundulus, with positive results.[34]

Apart from its role in melanogenesis, the functions of prolactin in fishes are little understood. Our most recent studies, reviewed below, indicate that it plays a major role in promoting the adaptation of *Fundulus heteroclitus* to survival in fresh water.[65]

CHROMATOPHORE HORMONES OF THE HYPOPHYSIS

There is an extensive literature on the chromatophore-regulating hormones of fishes, reviewed by Parker[53] and more recently by ourselves.[62] Fundulus has played a major part in numerous investigations. In this species, as in many teleosts, the melanophores are unresponsive to single injections of intermedin. In contrast to frogs, no melanophore dispersion can be observed, although chronic treatment, discussed in the previous section, leads to the eventual proliferation of newly formed melanocytes.[60] The lack of intermedin-dispersing sensitivity can be abolished, at least in Fundulus, by cutting the nerves which connect the affected area to the central nervous system, as shown by experiments with fish bearing Parker's so-called "faded denervated caudal bands."[51] The role of intermedin is apparently restricted, in Fundulus, to regulation of the number of melanophores in response to long-term background adaptation. It plays no part in rapid color changes which are under the control of the nervous system. However, it must be realized that each species of fish presents its own problem. In some, the melanophores are responsive to intermedin. In Fundulus, a combination of prolactin and intermedin is necessary for maintenance of normal melanin pigmentation, but Chavin[14] found that ACTH causes melanogenesis in xanthic goldfish whereas intermedin is ineffective.

Although the melanophores of Fundulus are not subject to the pigment-dispersing action of intermedin, xanthophore dispersion has been reported. Fries[28] and Matthews[45] used implants or extracts of whole pituitary glands of the same species, and it is therefore possible that intermedin was not the decisive factor. In our experiments on hypophysectomized Fundulus we have not observed yellowing after injection of purified intermedin, but no special study has been made of the problem, and a lack of response could be attributed to a reduction in the number of yellow pigment cells following the sexual regression of hypophysectomy. It will be recalled that androgens are necessary for the development of the nuptial coloration in males.

The hormonal responses of the leucophores (guanophores) and iridocytes are similarly in need of further investigation. The leucophores are white pigment cells containing guanin, or a guanin-like material, in amor-

phous form. The iridocytes, for which Fries[27] has proposed the noncommittal term 'antaugophores,' are iridescent white reflecting chromatophores in which the guanin-like material is in the form of flat hexagonal platelets. Odiorne[49] observed some dispersion of the guanophores in Fundulus following injections of Antuitrin. This material also caused melanophore concentration, which cannot be elicited in Fundulus by purified mammalian pituitary hormones,[42] and therefore the interfering action of unknown substances in the commercial solvent cannot be excluded. Fries[25, 27] has shown that hypophysectomy has no effect on the leucophores of *Cyprinodon variegatus,* a species closely related to Fundulus, nor on the antaugophores of the goby, *Bathygobius soporator.* Foster[22, 23] observed a color change in the iridocytes of Fundulus, from blue or green to orange or red, following treatment with Pituitrin (presumably containing intermedin). The change of color is attributed to a change in the layering of the guanin crystals, influencing the wave length of the reflected light. But, since dispersion of melanin granules in the melanophores is believed to be associated with sol-gel phenomena in the cytoplasm, the two types of response may be basically similar. It must be recalled, however, that Bagnara[2] has shown that in frogs the guanophores respond in the opposite manner to the melanophores: hypophysectomy causes a reduction in the number of melanophores and an increase in the number of guanophores; intermedin causes melanophore dispersion and proliferation, guanophore concentration and reduction. We have not observed guanophore proliferation in hypophysectomized Fundulus, but the abundance of guanin crystals, forming iridescent blue patches in the skin, is undoubtedly augmented after treatment of males with gonadotropins. This is correlated with androgen stimulation of the xanthophores and is a part of the vivid nuptial coloration.

The hypophysis of teleosts, but apparently not that of mammals, contains a melanophore concentrating hormone (MCH). Recent studies support the hypothesis that this substance is separable from intermedin.[42] The evidence strongly favors the view that MCH is associated with the anterior part of the pituitary, although a recent article by Kent[41] suggests that it may be formed there and stored in the intermediate lobe. Chemical studies by Imai[39] suggest that MCH is not a protein, peptide, lipid, or carbohydrate, but its nature was not determined. Our experiments on Fundulus have shown, among other things, that MCH can cause pallor not only in normal or hypophysectomized fish, but also in "denervated faded caudal bands" of dark background-adapted recipients. This experiment, like parallel experiments of earlier investigators with intermedin, excludes participation of the central nervous system.

NEUROHYPOPHYSIAL HORMONES

The teleostean pituitary is rich in posterior lobe hormones with oxytocic, vasopressor, and antidiuretic properties when tested on laboratory mammals.[62] However, the function of the neurohypophysial hormones in fishes remains obscure. They do not elicit renal antidiuresis in teleostean recipients, but the work of Sexton[71] indicates an enhancement of water uptake through the gills of the goldfish. This would parallel the situation in the purely aquatic clawed toad, Xenopus, in which posterior lobe hormones stimulate an increased water uptake through the skin not accompanied by renal antidiuresis.[20] Therefore in this species, as in teleosts, there is no increase in body weight resulting from water uptake plus water retention after neurohypophysial injections.

The experiments of Burden,[8] reviewed under Osmoregulation, failed to indicate that posterior lobe hormones played any direct role in enabling hypophysectomized Fundulus to survive in fresh water. If the findings of Sexton, cited above, can be applied to Fundulus, an increased water uptake through the gills could aggravate the physiological adjustment to fresh water.

With one exception, our studies of Fundulus have revealed no function for the neurohypophysial hormones of this species. The cytological studies of Sokol[77] showed that there is a marked depletion of neurosecretory material in the neurohypophysis of Fundulus during the breeding season. A correlation with reproduction may also be deduced from the discovery that injections of neurohypophysial hormones, including synthetic oxytocin, elicit a characteristic behavior syndrome which, for want of a better term, we have called the "spawning reflex."[79] The body is bent in an S-shaped curve and the fins vibrate rapidly, exactly as may be observed in the natural act of spawning. If the fish are ripe, the sex products may be emitted, but the response can be elicited equally effectively in immature or hypophysectomized recipients. Sex hormones, and a wide variety of mammalian anterior lobe preparations, were totally ineffective. The only exception was an LH that clearly contained some posterior lobe activity.

HEMATOLOGY

Dr. Anna M. Slicher, working in the Bingham Oceanographic Laboratory, has made a careful study of the normal hematology of Fundulus; various hormonal influences were also investigated.[74, 75, 76] During the breeding season the red cell count is higher in males than in females, as in mammals after puberty. In winter, during the period of sexual regression, both sexes revert to a common intermediate value: the red cell count rises

a little in females and drops significantly in males. This cyclical sex difference is well known in a number of teleostean species, but proof that it was caused by the sex hormones was lacking. Dr. Slicher showed that chronic treatment of hypophysectomized males with methyl testosterone restored the red cell count to normal breeding levels.

The effects of hypophysectomy, which had not been previously investigated, indicated changes parallel to those known in hypophysectomized laboratory mammals: in general, there is a state of pancytopenia. Particular interest attached to the total disappearance of circulating eosinophils. It seems probable that they are stored in the tissues, since an injection of saline evoked their transitory re-appearance in the blood stream two hours later.

A major aspect of Slicher's investigations concerned the response to injections of ACTH. She had previously discovered[78] that in another teleost, Tilapia, this hormone caused leucopenia at low doses and leucocytosis at higher doses. In Fundulus a single high dose caused a six-fold increase in the white cell count two hours after injection; a similar but lesser effect was observed in hypophysectomized recipients. The response of Fundulus to low doses of ACTH was not investigated, but it was found that cold shock, which elicits endogenous release of ACTH in Astyanax,[97] resulted in leucopenia after one hour, followed by leucocytosis after two hours. Chronic treatment of hypophysectomized recipients with α_s=adrenocorticotropin at a physiologically moderate dose restored both red and white cell counts to normal levels and elicited the re-appearance of circulating eosinophils, still present 24 hours after the last injection.

A single injection of cortisol, the naturally occurring glucocorticoid of Fundulus,[58] has essentially the same effect as ACTH on the blood of hypophysectomized recipients: little change in the red cell count and a moderate increase in the white cell count. These primary effects of cortisol on the hemopoietic tissues are masked, in normal fish, by interractions that evidently involve the pituitary. Leucopenia was observed, instead of leucocytosis; eosinopenia, instead of the re-appearance of these cells in the blood stream. Further studies of the problem in normal fish indicate that the nature and magnitude of the response may depend on the sex and on the stage of the reproductive cycle.

As noted above, either androgen or ACTH can restore the red cell count of hypophysectomized Fundulus to normal levels, if administered over a period of time. It was also found that TSH, at physiological levels, can cause an increase in the red cell count. On the other hand, prolactin, intermedin, and growth hormone had no significant effect.

OSMOREGULATION

Dr. Charles E. Burden,[8] at that time a senior undergraduate majoring in Zoology at Yale, demonstrated that hypophysectomized *Fundulus heteroclitus* cannot be kept in fresh water. Death results from progressive asthenia which develops slowly and usually becomes critical in about 6-7 days at 15° C. Titrations of blood chloride revealed severe hypochloremia. In normal Fundulus at 15° C. there was no difference in serum Cl between fish kept in salt water and those which had been kept for 8 days in fresh water: the average for both groups was 0.886 g% NaCl. Hypophysectomized fish in salt water showed essentially similar levels, on the average 0.817 g% NaCl, but, at the onset of severe asthenia, hypophysectomized fish in fresh water had only 0.322-0.451 g% NaCl.

Burden investigated a variety of mammalian hormones in an attempt to identify the agent responsible for promoting survival in fresh water. Negative results were obtained by replacement therapy with TSH, thyroxine, ACTH, desoxycorticosterone acetate (DOCA), growth hormone, and a combination of TSH, ACTH, and GH. With the exception of the rather high dose of DOCA, the hormones were administered at physiological levels. Hog posterior lobe powder was also ineffective. On the other hand, a brei of Fundulus pituitary glands was completely successful, and a brei of perch pituitary was partially effective. Pollack pituitary was ineffective, and Burden suggested that the hypophysis of freshwater and euryhaline teleosts might contain a special hormone promoting survival in fresh water.

Burden also made some interesting observations on gill cytology. He found that the number and condition of the so-called "chloride cells" was unaffected by hypophysectomy, and that they showed similar changes to those reported for normal fish when transferred from salt to fresh water.[4, 15] Failure of "chloride cell" function is therefore not responsible for the hypochloremia of hypophysectomized Fundulus in fresh water. On the other hand, atrophy of the mucous cells of the gills was observed in such fish, raising the question of their possible protective role in osmoregulation.

Burden's negative results with TSH and thyroxin were supported by the work of Patricia J. Harris[36] who employed hypothyroid Fundulus. Hypothyroidism was the result of treatment with radioactive iodine, and the fish were carefully screened with tracer doses of I^{131} before being assigned to the freshwater experiment. Harris was unable to detect any difference between hypothyroid and euthyroid fish in respect to survival in fresh water. None of the fish was completely athyroid, but certainly severe hypothyroidism has no detrimental effect on freshwater adaptation. Contrary to Burden, she found that blood chloride was lower in fresh than

in salt water, irrespective of the condition of the thyroid. Her experiments were made at a higher temperature than his, 20° C. as compared with 15° C., and the fish were kept for a longer period in fresh water, 16 days compared with 8 days. Either or both of these factors may have influenced the change in the internal medium. It should be noted that both Garrard[28] and Bergeron[4] demonstrated the ability of *Fundulus heteroclitus* to maintain the osmotic pressure of the blood at a constant value in either salt or fresh water. However, this does not preclude a change in the ratio of bicarbonate to chloride. In other species of fish it is known that the buffering capacity of the blood is greater in fresh than in salt water.[11, 18, 21]

The problem of failure of hypophysectomized *F.heteroclitus* to survive in fresh water has been subjected to further investigation. Recent studies in the Bingham Laboratory have gone far to clarify the hormonal aspects of the problem.[65] Confirming the findings of Burden with ACTH and DOCA, we were able to exclude direct participation of the adrenal cortex in promoting freshwater survival. Neither the natural glucocorticoid, cortisol, nor aldosterone, administered at physiological levels, had any beneficial action, and extracts of head kidney containing the adrenal cortical tissue of Fundulus were equally ineffective. An extract of carp corpuscles of Stannius was wholly ineffective, although histological investigations after experimental treatments have implicated this gland of unknown function in teleostean osmoregulation.[68] The possibility that renin, supposedly present in the kidneys of freshwater but not of marine teleosts,[24] was a factor in freshwater survival was also excluded and, in a recent unpublished experiment, similar results were obtained with parahormone. A brei of whole rat pituitary, on the other hand, was partially effective. The active agent is probably the lactogenic hormone, since injections of highly purified prolactin were found to enable hypophysectomized fish to survive in fresh water. Although the recipients appeared lively and active, even after 20 days, they did not eat well. Complete adaptation to fresh water may require the simultaneous administration of prolactin and some unidentified pituitary hormone. The possibility that fish prolactin might be more effective than sheep prolactin must also be considered. A search for prolactin, among the many partially purified fish pituitary fractions prepared by Dr. Alfred E. Wilhelmi, must now be undertaken.

The foregoing discussion deals only with the special problem of pituitary control of osmoregulation in Fundulus. The presence of the hypophysis is not necessary for eels to survive either in fresh or salt water, and both marine and freshwater teleosts can be successfully maintained in their appropriate environment after removal of the pituitary.[62]

CALCIUM METABOLISM AND THE SUPPOSED ABSENCE OF THE PARATHYROID

Rasquin and Rosenbloom[70] suggested that the ultimobranchial body might be the homologue of the mammalian parathyroid gland in teleosts. The evidence rested on hypertrophy of this tissue, correlated with skeletal abnormalities and a general state of panhypopituitrism in Astyanax that had been kept up to two years in total darkness. Rampone, in Hoar,[37] could find no changes in bone or muscle ash of goldfish that had been treated with parahormone, but Budde[7] obtained more encouraging results with *Lebistes reticulatus*. Although no histological changes in the bone could be observed after short periods of treatment, longer periods resulted in bone resorption and prolonged treatment led to excessive bone deposition.

We have observed that there is a disturbance of calcium metabolism in Fundulus, following removal of the pituitary. This is reflected in the frequent incidence of carbonate apatite renal calculi,[57] and in defective calcification of new growth on the scales, elicited by treatment with growth hormone.[59] Only fish which showed total absence of all traces of a pituitary remnant exhibited these overt symptoms.[62] Plans for a further study of this problem are now in progress.

ISLETS OF LANGERHANS

The exocrine pancreas of Fundulus is of the diffuse type, with ramifications that follow the branches of the hepatic portal vein within the liver. The endocrine tissue is restricted to one or two compact nodules located in the vicinity of the gall bladder. Isletectomy, which has been performed on other teleostean species,[44] is therefore possible, but the operation is difficult owing to the danger of severing ductules of the exocrine pancreas. Our first attempt resulted in autodigestion of the viscera and we have not returned to a study of methods for perfecting the surgical procedure.

The functions of the islet tissue in fishes appear, from the literature,[37] to be the same as in higher vertebrates. We have made no study of this problem in Fundulus but may mention, in passing, that neither hypophysectomy nor administration of beef growth hormone had any apparent effect on the cytology of the alpha and beta cells which are recognisable by standard staining procedures in this species.[57, 58]

GASTRO-INTESTINAL HORMONES

Although some work has been done on elasmobranchs, almost nothing is known about the possible hormonal regulation of digestive secretions in teleosts.[37] Secretin was early identified in the upper intestinal mucosa of salmon,[8] but many species of fishes, like Fundulus, have no stomach and

there is no acid phase of digestion. The release of pancreatic secretion cannot, therefore, be regulated by the discharge of secretin in response to the passage of acid from the stomach into the duodenum. It is possible that nervous, rather than hormonal pathways are involved in such species.

UROHYPOPHYSIS

A recent abstract by Holmgren[38] on the cytology of the caudal neurosecretory organ of Fundulus should be noted. This peculiar organ, a storage-release center at the end of the spinal cord, has been described by Enami[19] in many species of fishes, and endocrine functions are suspected. Holmgren, following Enami, suggests a possible role in osmoregulation, but no experimental evidence was presented.

CONCLUSION

Endocrinological studies on *Fundulus heteroclitus* confirm the hypothesis that, in many respects, the hormonal mechanisms of fishes resemble those of mammals. However, the physiological peculiarities of poikilotherms, and the special requirements of life in an aquatic environment that may be either hypo- or hypertonic to the internal medium, impose unfamiliar problems. The functions of the thyroid, adrenal, and neurohypophysial hormones are not well understood in fishes. The apparent absence of the parathyroid and the unexpected functions of the lactogenic hormone (prolactin) offer new ground for future research. In the latter case we are confronted with an outstanding example of evolutionary changes in the target organ of a well-known pituitary hormone. On the other hand, the melanophore-concentrating hormone which, unlike intermedin, is apparently absent in the mammalian hypophysis, provides an example of a basic evolutionary change in the hormonal secretion of the so-called "master gland."

ACKNOWLEDGMENTS

The foregoing review has been concerned with exploratory work of the author and her collaborators on a single teleostean species, *Fundulus heteroclitus*. Lack of space precluded more than passing reference to the pioneer work of many leading investigators in the field of fish endocrinology. Names such as those of Professor Bernardo Houssay, Professor Maurice Fontaine, Professor I. Chester Jones, Dr. William Hoar, Dr. N. L. Gerbil'skii, Professor Aubrey Gorbman, and many others, too numerous to mention, come readily to mind. Finally, in closing, I should like to express my indebtedness to Professor Alfred E. Wilhelmi without whose continued interest and frequent collaboration it would have been impossible to advance these studies to a point where it is hoped that they may provide a lasting contribution to the body of scientific knowledge.

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ISOLATION OF CORTISOL FROM A PHEOCHROMOCYTOMA‡

Although the adrenal cortex and medulla arise from different embryological tissues, they are intimately associated in the developing embryo. The adrenal cortex arises from splanchnic mesenchyma, while the medulla arises from nervous tissue.[14] The chromaffin cells migrate by way of the celiac plexus to penetrate the adrenal cortex as scattered islands which later aggregate to form the adrenal medulla. It is conceivable that islands of chromaffin cells surrounded by adrenocortical cells might develop as aberrant tissue. Later, if a tumor developed in this aberrant tissue, both adrenocortical and medullary elements would be present.

Clusters of cells which resemble cortical cells are occasionally seen in pheochromocytomas.[7, 9, 10] Reports in the literature suggest that certain pheochromocytomas do secrete steroids. A striking case reported in 1942, by Neff and co-workers,[12] was that of a 16-month-old-girl with marked hirsutism, overdevelopment of external genitalia, obesity, and paroxysmal hypertension. Removal of an adrenal tumor from this patient resulted in an immediate fall in blood pressure and regression of all symptoms. Histologically, the tumor was a pheochromocytoma, with one peculiar microscopic feature: lipoid material within many cells.

Chenualt, *et al.*[6] described a patient with paroxysmal hypertension, diabetes mellitus, elevated urinary 17-hydroxycorticoid excretion, and an abnormally increased excretion of 17-hydroxycorticoids after an ACTH test. Symptoms and chemical findings returned to normal after removal of a tumor at the bifurcation of the aorta which contained 7.5 mg. of catecholamines per gm. of tumor with more than 6.2 mg. as norepinephrine.

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‡ This investigation was supported in part by Grant #C-3998(C) and Grant #A-254 (C5) from the Division of Grants, National Institutes of Health, U.S. Public Health Service.
Received for publication April 1, 1959.

In the present study, slices of a pheochromocytoma have been incubated and a compound which resembles cortisol has been isolated from the incubation media.

CASE HISTORY

A 62-year-old white female entered the Memorial Unit of the Grace-New Haven Community Hospital for surgical removal of a right suprarenal mass. About 18 months prior to admission, because of left flank pain, an intravenous pyelogram was performed which revealed normal kidneys and a right suprarenal mass. The pain subsided spontaneously without a definitive diagnosis of the suprarenal mass. During the year prior to admission she had a 10-lb. weight loss despite a good appetite. The patient noted easy fatiguability and two to three soft bowel movements a day. Two months prior to admission a GI series and barium enema were negative, while an intravenous pyelogram revealed the suprarenal mass to be unchanged in size or shape. The patient denied any episodes of weakness, nervousness, sweating, palpitations, or hypertension. Her private physician reported her blood pressure was normal on several occasions prior to hospitalization.

Physical examination was unremarkable except for an elevation of blood pressure on two occasions—150/100, and 170/100. Multiple other blood pressures were normal. Laboratory studies revealed a normal urinalysis and hemogram, NPN 34mg.%, FBS 109%. Serum electrolytes are represented in Table 1.

The patient had none of the signs or symptoms of an adrenocortical or medullary tumor. The low serum potassium raised the question of an aldosterone-secreting tumor. However, it was considered that the frequent bowel movements probably accounted for the potassium deficiency.

The patient's reluctance to allow diagnostic procedures prevented further investigations. At surgery on the fourth hospital day a large right adrenal mass was discovered. Despite vigorous manipulation, no significant alteration in blood pressure occurred. After the tumor was dissected free, and prior to clamping of the large veins draining the tumor, the blood pressure dropped precipitously. Vasoxyl®, levophed®, blood, and 200 mg. of hydrocortisone hemisuccinate were administered within a short period. The blood pressure rose to 100-110/70. For several days postoperatively the patient was lethargic, weak, and hypotensive unless maintained on levophed®. Cortisone and desoxycorticosterone were also administered but probably without significant effect. Gradually improvement occurred with stabilization of the blood pressure at 110/70. The serum potassium re-

turned to normal. Several months postoperatively the patient was reported to be doing well.

PATHOLOGY

Gross. The tumor was a 169-gram, encapsulated mass. The right adrenal was attached to one side, but separate from the tumor.

Microscopic. Along one surface is the capsular area beneath which is a narrow layer of finely vacuolated adrenal cortical cells with small oval nuclei and the general

TABLE 1.

	Serum values			
Date	*CO_2 mm/L*	*Cl= meq/L*	*Na+ meq/L*	*K+ meq/L*
12/3/57	21.8	101	145	2.9
12/5/57	27.0	98	146	2.8
12/11/57*	25.0	96	141	5.5

	Urine values		
Date	*17 ketosteroids mg/24 hr.*	*17 ketogenic steroids mg/24 hr.*	*catechol amines µg/24 hr.*
12/2/57	4.5 (5-15)**	8.3 (8-20)**	616 (38-158)**

* Postoperative (surgery on 12/6/57).
** Normal range.

configuration of zona glomerulosa. Beneath this zone there is a large mass of highly vascularized tumor tissue. Many blood-filled spaces are lined by tumor cells which in some areas extend to the capsule.

Figures 1 and 2 are high power views of the tumor cells which illustrate the large size of the cells compared to the erythrocytes in the vascular spaces. They have abundant, finely granular cytoplasms which vary with hemotoxylin-eosin stains from pink to deep red purple. Many cells contain several large, vesicular nuclei with sharp nuclear membranes and prominent nucleoli. Mitoses are absent. A small proportion of cells present an unusual feature for a pheochromocytoma. These viable, well staining cells contain numerous vacuoles within their cytoplasm suggestive of lipoid material (Figs. 1, 2). A Sudan IV lipid stain of frozen and later formalin-fixed tumor tissue illustrates a few cells which contain lipid-staining material. The spotty distribution of these cells is approximately similar to those with clear vacuoles.

Foci of scarring and lymphocytic infiltration are present in some areas. The blood vessels contain numerous hemosiderin-filled macrophages. Fine brown pigment granules as well as coarser granules characteristic of hemosiderin are present in many tumor

cells. Although chromaffin stains are negative, not all of the brown pigment is positive for iron stains.

METHODS

The excised tumor was freed of fat and weighed. Tissue slices were made with a Stadie-Riggs microtome. The tissue was incubated in Krebs-Ringer bicarbonate buffer with 200 mg.% glucose in an atmosphere of 95% O_2, 5% CO_2 for 3 hours. Since it was unclear at the time of surgery whether this was a nonfunctioning adrenal tumor, pheochromocytoma, or adrenal cortical tumor, care was taken to avoid incubating any part of the tumor's capsule which might contain adrenocortical tissue. Sections of tumor tissue for incubation were taken from the central portion of the tumor, well away from the yellow speckled few millimeter thick capsule. The rest of the tumor was stored at minus 20° C. Slices of the excised right adrenal cortex were also incubated in separate flasks.

After incubation, the media from the tumor and adrenal cortex were extracted separately with redistilled chloroform. The chloroform extracts were washed once with 1/10 volume of 0.05N NaOH, twice with 1/10 volume distilled water, dried with anhydrous sodium sulfate, and evaporated *in vacuo* at 40° C. The residue was applied to a paper chromatogram in a modification of the toluene-propylene glycol system[4] along with reference corticosteroids. The standards and unknown compounds were located by the ultraviolet scanning technique. After elution with ethanol, the unknown ultraviolet absorbing band was rechromatographed in the Bush-C system[5] with appropriate standards on either side. Standards and unknown were located as before. Chemical characteristics of the compound were determined after elution from the second chromatographic system. Fluorescence in alkaline solution was measured by the method of Abelson and Bondy[1]; Porter-Silber reaction[17] and appropriate paper blanks were measured simultaneously.

Acetylation was carried out with 20 lambda each of redistilled acetic anhydride in anhydrous benzene (15% V/V) and redistilled anhydrous pyridine for 24 hours. Chromatography of the acetylated compound was performed in the carbon tetrachloride-methanol-water system[15] with standard cortisol acetate. Radioactive progesterone-16-H3 with specific activity 558mC/mM was obtained from the New England Nuclear Corporation. Chromatography of an aliquot with carrier progesterone revealed it to be at least 98 per cent pure. Radioactivity was counted in a liquid scintillation counter, where the efficiency for tritium counting was 15-16 per cent.

Urinary 17-ketosteroids and 17-ketogenic steroids were measured by the method of Norymberski, *et al.*[18] Urinary catechol amines were determined by the method of Helmer.[8] The catechol amine content of the tumor was estimated by a modification of Von Euler and Floding's method.[18]

CLINICAL STUDIES

Table 1 presents results of the clinical studies. A specimen of urine collected and frozen preoperatively and analyzed several months later revealed low normal 17-ketosteroids, 17-ketogenic steroids, and elevated catechol amines. Serum potassium concentrations were low preoperatively.

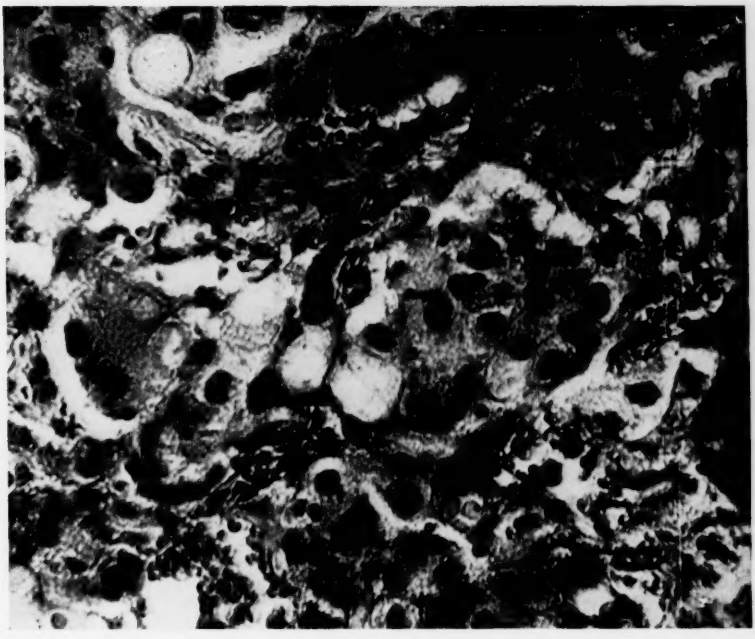

FIG. 1. Representative section of tumor, showing abundant granular cytoplasm, vesicular nuclei, prominent nucleoli and scattered cytoplasmic vacuoles. Hematoxylin and eosin stain. x645.

FIG. 2. Section of tumor, showing variability of staining, tendency to elongation, multinuclear giant cells, and occasional vacuolization of cytoplasm. Hematoxylin and eosin stain. x645.

More than 3.0 mg. per gram of tissue of catechol amines were found in the tumor.

IN VITRO STUDIES

Table 2 illustrates chromatographic and chemical characteristics of the compound isolated from the incubation media of 4 grams of tumor slices. After 2 chromatograms approximately 6 ug. were found. Mobilities are expressed as ratios of the mobility of the compound to that of standard compound E (cortisone). The compound had the same mobility as stand-

TABLE 2. FRESH TUMOR INCUBATION

	Standard F	*Tumor "F"*
Toluene-propylene glycol	R_E 0.45	R_E 0.41
Bush C	R_E 0.67	R_E 0.69
Ultraviolet absorbtion	+	+
Alkaline fluorescence	+	+
Porter-Silber	+	+

Chromatographic and chemical characteristics of the "cortisol" (F) isolated from the media. R_E refers to the ratio of the mobility of the compound, or standard cortisol to standard cortisone.

ard compound F (cortisol) in the toluene-propylene glycol system, and after rechromatography in the Bush C system. This compound absorbed ultraviolet light, fluoresced in alkaline solution and gave a positive Porter-Silber reaction.

Could this finding of cortisol be the result of inadvertently incubating small amounts of adrenocortical tissue that might have invaginated into the center of the tumor? In an attempt to exclude this possibility, a second incubation was carried out several months later. The frozen tumor was thawed. Approximately 5 grams of tissue were cut from the center of the tumor, blotted dry, and incubated. Any section containing large blood vessels or grossly evident connective tissue was discarded. Each flask contained the constituents as outlined in Table 3. TPN^+ and glucose-6-phosphate were added since Kortiz and Peron[11] had shown frozen rat adrenals in the presence of TPN^+ and glucose-6-phosphate produced more corticosteroid than normal adrenals. In addition, Bongiovanni[8] demonstrated that homogenates of frozen human adrenals obtained at surgery or autopsy were capable of producing corticosteroids in the presence of appropriate

cofactors. After incubation each section of tumor was examined microscopically. No obvious adrenocortical tissue was seen. Sections of similarly frozen and thawed adrenal tissue showed easily identifiable cortical tissue. However, since such frozen tissue is considerably distorted microscopically, it cannot be unequivocally stated that no adrenal cortical tissue from the capsule was present.

Table 4 represents the data from the extract of the incubation media. Approximately 10 ug. of a compound similar to cortisol was detected. It had the same mobility as standard cortisol in toluene-propylene glycol and Bush C systems. After elution from the Bush C chromatogram, this com-

TABLE 3. INCUBATION SYSTEM

Substrate	*Per flask*
Tumor	600 mg.
Progesterone-16-H^3	1.89×10^6 DPM 0.5 μg.
Gluc.-6-Phosphate	10 mg.
TPN	10 mg.
Buffer	10 ml.

pound was acetylated and rechromatographed in a carbon tetrachloride, methanol-water system. This acetylated compound had the same mobility as standard cortisol acetate and contained a small amount of radioactivity. This suggests that a portion of the radioactive progesterone was hydroxylated by the tumor slices to form cortisol. However, this was only a minute fraction of the total radioactivity. This low amount of incorporation might possibly be due to precursors within the cells being more readily hydroxylated to cortisol than tracer quantities of progesterone added to the media. The progesterone might not easily gain access to the small number of "adrenocortical-type cells" interspersed throughout the relatively large amount of tumour tissue. Another possibility is that endogenous cortisol had diffused into the tumor from adjacent adrenocortical tissue prior to incubation. This latter possibility seems unlikely since the content of cortisol within the adrenal is quite low. A sufficient concentration gradient would hardly be present for cortisol to diffuse into the center of this large tumor.

It appears therefore that the enzymes necessary to convert tritiated progesterone to tritiated cortisol were present in this tissue. However, since the yield of tritiated cortisol was so small the possibility of radioactive

impurities cannot be excluded, although similar studies with control tissues from another species would not indicate this.[10]

Table 5 compares the *in vitro* release of the patient's right adrenal cortex and the pheochromocytoma. The third column illustrates that the micrograms of cortisol produced per gram of tissue is much higher for the adrenocortical tissue. However, although the adrenal cortex was not weighed, if it is assumed that it was a large 7-gram gland, and that the production of steroids by the rest of the tumor tissue was at least that of the incubated tissue, the total steroid production of the adrenal cortex is less than that of the tumor.

TABLE 4. FROZEN TUMOR INCUBATION

	Standard F		*Tumor "F"*
T/PG	R_E 0.53		R_E 0.55
Bush C	R_E 0.65		R_E 0.62
		Acetylation ↓	
C Cl_4/M	$R_{Fac.}$ 1.00		$R_{Fac.}$ 1.01 DPM = 125

Chromatographic mobilities of the radioactive "cortisol" (F). R_E determined similarly to R_E in Table 2. $R_{Fac.}$ refers to the ratio of the mobility of the acetylated derivative of the radioactive "cortisol" to authentic cortisol (F) acetate which was arbitrarily assigned $R_{Fac.}$ 1.00. DPM means disintegrations per minute.

DISCUSSION

Tumors of the adrenal gland are frequently difficult to classify histologically. Adrenocortical adenomas may resemble pheochromocytomas. Pheochromocytomas sometimes contain clusters of cells which resemble adrenocortical cells. Without positive chromaffin stains, Karsner[9] considers the histological diagnosis of pheochromocytoma unsafe. Obviously a tumor composed of both adrenocortical and medullary elements would be most difficult to diagnose histologically.

The value of the incubation technique as an adjunct to the pathology of hormone-secreting tumors was demonstrated by Baggett, *et al.*[3] in the case of a testicular tumor which histologically resembled a large, undifferentiated testicular tumor. Incubation studies revealed that the tumor tissue could convert C-14 acetate to cortisol and therefore was probably aberrant adrenocortical tissue.

In the present case, it appears that a tumor which resembled a pheochromocytoma histologically and contained an excess of catechol amines, released cortisol *in vitro*. This was correlated with the appearance of clear vacuoles within some tumor cells suggestive of lipid. There are reports in the literature[6, 12] which indicate that pheochromocytomas may also secrete adrenocortical steroids. The clinical picture would depend upon the type and quantity of steroid produced. Perhaps part of the circulatory collapse sometimes following surgical removal of a pheochromocytoma is due to removal of a steroid-producing tumor. This tumor might be producing a quantity of steroid sufficient to inhibit ACTH secretion, yet insufficient to

TABLE 5. A COMPARISON OF THE *IN VITRO* STEROID RELEASE OF THE RIGHT ADRENAL CORTEX AND THE PHEOCHROMOCYTOMA

Specimen	*Total weight gm.*	*F μg./gm. tissue*	*F total μg.*
Adrenal	?	21	(147)
Tumor	169	1.3	219

produce symptoms of hyperadrenalism. The removal of this tumor would leave atrophied adrenals to cope with the stress of surgery.

This did not appear to be the situation in the present case, since the hypotension did not appear to respond to hydrocortisone alone. Actually, the nature of the vascular collapse was unclear. Such a clinical state would be difficult to detect prior to surgery. *In vitro* studies would help to clarify this possibility. Moreover, it would be of interest to determine whether adrenocortical tumors associated with Cushing's syndrome contain an excess of catechol amines.

SUMMARY

A 169-gram adrenal tumor was removed from a relatively asymptomatic 62-year-old woman. Histologically, the tumor resembled a pheochromocytoma yet contained vacuolated tumor cells suggestive of lipid. Assay of the tumor revealed an excess of catechol amines. Slices of tumor tissue released a compound *in vitro* which had the chromatographic characteristics of cortisol.

The implications of these findings are discussed.

ACKNOWLEDGMENT

The authors wish to thank Dr. N. Giarman and M. G. Golden for measuring catechol amines in the tumor; Dr. Walter Herrmann for measuring urinary 17-ketosteroids and ketogenic steroids; Dr. Max Taffel for his generous cooperation; Dr. Philip K. Bondy for his advice, criticism, and encouragement throughout the course of these studies; and A. Kuljran for technical assistance.

ADDENDUM

A somewhat similar case has been brought to our attention by Dr. John Beck of the Royal Victoria Hospital, Montreal, Canada, who has kindly sent us a summary of the case. The patient's chief complaint was seizures. She was found to have a markedly fluctuating blood pressure and a pale left optic disc. Laboratory studies revealed elevated plasma and urinary catecholamines, positive regitine test, abnormal glucose tolerance test, compression fractures of several vertebrae, and a right suprarenal mass. On September 5, 1958, an adrenal tumor was removed which histologically was compatible with a pheochromocytoma, although chromaffin stains were negative. Postoperatively, she had marked hypotension which only responded to a combination of norepinephrine and cortisol. The urinary catecholamines and glucose tolerance returned to normal.

Incubation of the adrenal tissue and tumor tissue separately revealed that both produced significant quantities of steroids, and both were able to convert desoxycorticosterone to corticosterone and cortisol. Neither showed a clear response to ACTH *in vitro*.

An extremely interesting feature of this case was the markedly diminished excretion of 17-ketosteroids and Porter-Silber corticoids pre- and postoperatively. Urinary gonadotrophins were 105 mouse units. Postoperatively repeated ACTH administration produced appreciable increases in steroid excretion. Further studies of the patient's adrenocortical function are in progress.

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MEMBRANES, TAGGED COMPONENTS, AND MEMBRANE TRANSFER COEFFICIENT**

Concepts, introduced into physics in the analysis of the Peltier effect by Thomson in 1854 and generalized by Onsager in 1931, have made it clear that the equations commonly used for the description of membrane transfer processes are incomplete. Kedem and Katchalsky[6] have recently emphasized the utility of these concepts in removing inconsistencies of interpretation introduced by the use of incomplete equations for the description of the movement of water through membranes. Incomplete equations are also used in present accounts of the behavior of tagged species in transfer systems, which also give rise to interpretive difficulties. A good example of the nature of some of the difficulties encountered is illustrated by the classical investigations of Hevesy, Hofer, and Krogh.[5] They had occasion to measure the over-all transfer coefficient for the absorption of water through the skin of a frog. They found to their surprise that two values of the transfer coefficient of water for which they could not give a satisfactory explanation were obtained. The value of the transfer coefficient depended upon how the experiment was performed. The transfer coefficient estimated from the weight change of the frog in an osmotic experiment was some five-fold greater than that determined from an appropriate experiment with tagged water. Furthermore, they observed that the flux of tagged water appeared to be independent of the flux of normal water. Though they made many suggestions attempting to account for these experimental findings, they concluded that further information was needed before the experimental results could be 'explained.'

Similar experimental results have been obtained many times since in investigations conducted on membranes of living systems. Some investigators have regarded the two transfer coefficients to be the result of some biological artifact, such as incomplete mixing when tagged water is used, or "active secretion" through the membrane. The experimental results obtained by Swanson[15] and by Mauro[7] on artificial membranes rendered

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** Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

Received for publication March 18, 1959.

improbable such possible explanations. These investigators found ratios of the transfer coefficient determined by osmotic methods to transfer coefficient determined with tagged water to be from 25 to several hundred fold. It will be demonstrated that such behavior is to be expected when the complete equations for transfers at membranes are applied.

The theory of coupled steady-state processes provides an adequate explanation of such experimental results and allows one to bring the description of transfer processes of tagged species at membranes into agreement with the general theory of all transfer processes. Much of the theory stems from the investigations of Onsager,[4, 8, 9] and a general treatment of all irreversible processes is given by de Groot.[3] The purpose of the present report is to give an outline of the theory as applied to membranes in as elementary a form as possible in order to make clear the concepts employed and the approximations that are made. Particular attention will be paid to the behavior of tagged materials at membranes. To keep the treatment elementary, only nonionic substances in isothermal systems will be considered.

GLOSSARY OF TERMS

α—acceleration
A—effective area of membrane, cm^2
C—concentration, number of kinetic units per cm^3 or $mols/cm^3$
D—Diffusion coefficient, cm^2/sec
d—infinitesimal difference
del—gradient operator
Δ—finite difference
F—driving force
F—Gibbs' free energy function
f—frictional coefficient
h—permeation coefficient, cm/sec.
k—time constant, reciprocal seconds
J—generalized flux
λ—conductance coefficient
L—equivalent length
ln—natural logarithm
M—an element of the admittance matrix, cm/sec.
M—mass
N—species mol fraction, or actual mol fraction
n—number of kinetic units or number of mols
P—hydrostatic pressure, $dynes/cm^2$
p—escaping tendency or fugacity
p—vapor pressure
π—osmotic pressure $dynes/cm^2$
R—gas constant 8.314×10^7 ergs per mol per degree
r—effective radius of a pore, cm
Σ—summation sign
T—temperature, degrees absolute
t—time, sec.
μ—potential or Gibbs chemical potential function
V—volume of membrane cm^3
$\bar{v}$—partial molar volume cm^3/mol
v—velocity
Y—resistive force
Z—a conversion factor

Subscripts

e—exchange
i—a particular component
j—any component except i
k—any component
s—solute
w—water
1—species 1
2—species 2

THEORY

General considerations. From the principals of elementary rational mechanics, we will write for the equation of motion of a kinetic unit of mass M_i in viscous medium the following relation:

$$F_i = M_i a_i + Y_i \tag{1}$$

If the accelerations a_i in the system are 0 or second order, then the system can be said to be in a steady state and

$$F_i = Y_i \qquad \text{(steady state)} \tag{2}$$

When the migration velocity of the kinetic unit is small, the resistive force Y_i becomes linear in the velocity, and

$$F_i = f_i n_i v_i \quad (v_i \text{ sufficiently small}) \tag{3}$$

If there are several components in motion through the membrane, two possibilities arise. The transfer of the individual components through the membrane can take place at different sites in such a way that no interference of the motion of one component with the motion of another component takes place. Such a membrane could well be called a mosaic membrane, in which case all that would be needed to describe the system would be a set of equations of the form of 3 for each component in motion and a mosaic element of the membrane for each component present. A second possibility is that the motions of the components through the membrane interfere with one another. This possibility is the more representative of transfer processes through membranes in general and certainly must be the possibility when two species of the same substance are present. Let us represent this situation by a resistive force made of several parts, a contribution from each of the components in motion, or

$$F_i = Y_i - \sum_j Y_{ij} = f_{ii} n_i v_i - \sum_j f_{ij} n_j v_j \tag{4}$$

The negative sign in the front of the summation sign is due to the fact that the v's are vector quantities, and if v_i and v_j are in opposite directions, the resistive force to the motion of the 'i'th kinetic unit is greater than when v_i and v_j are in the same direction. The f's therefore are positive quantities. The velocities in equation 4 are to be taken with respect to the membrane. We can express n_k in a variety of units, such as number of grams, number of mols, or number of cc., for example. The frictional coefficient must then be expressed in corresponding units, such as the specific frictional coefficient, the molar frictional coefficient, or the volume frictional coefficient respectively.

If we now define a quantity, J_i, the flow of the kinetic unit per unit area of the membrane, as the flux, we recognize that

$$J_i = dn_i/Adt = \frac{n_i}{V} v_i = C_i v_i \tag{5}$$

where C_i is the concentration of the kinetic unit in the membrane and V is the volume of the membrane. Dividing both sides of equation 4 by V and replacing the C_v terms by the J's we obtain

$$F_i/V = f_{ii}J_i - \sum_j f_{ij}J_j \tag{6}$$

We now assume that the driving force F_i can be derived from a gradient of a potential, or that the F_i forces are conservative. The term F_i/V would then be given by

$$F_i/V = -\frac{n_i}{V} \mathrm{del}\mu_i = -C_i \mathrm{del}\mu_i \tag{7}$$

Substituting this quantity into equation 6 and solving equation 6 for the J's we obtain

$$-J_i = \sum_k \lambda_{ik} C_k \mathrm{del}\mu_k \tag{8}$$

where the λ's are the functions of the f's. If we let

$$M_{ik} = \frac{\lambda_{ik} C_k}{Z} \tag{9}$$

and

$$X_k = Z\mathrm{del}\mu_k \tag{10}$$

then we can write equation 8 in the general form

$$-J_i = \sum_k M_{ik} X_k \tag{11}$$

In equation 11 we designate J_i as a generalized "flux," X_k as a generalized "force," and M_{ik} as an element of the admittance matrix (see reference 4).

Whereas our derivation of equation 11 would admit the expression of the generalized "forces" and the generalized "fluxes" in a variety of units, our choice can always be made in accordance with thermodynamic considerations. In any system in which spontaneous fluxes are present, the Gibbs' free energy of the system must decrease with time. This requires that

$$-dF/Adt = \sum_k J_k X_k \tag{12}$$

or the sum of the products of the *conjugate* "forces" and "fluxes" must have the dimensions of a change in energy per unit area of the membrane per unit time. If we choose to express J_k in the units of number of mols crossing a unit area of a membrane in a unit time, then the conjugate "force" X_k becomes the gradient of the Gibbs' chemical potential function times Z.

These are the conjugate variables we will use in subsequent discussion, and in this instance the value of Z in equations 9 and 10 is one cm. Other pairs of conjugate variables might have been selected. The appropriate choice is largely dictated by the demands of the particular problem.

Onsager has demonstrated that when the J's and X's are conjugate variables, simple relations exist among the cross coefficients of equation 11. He shows that

$$M_{ik} = M_{ki} \tag{13}$$

for all generalized "forces" except those derived from magnetic or Coriolis forces, which need not concern us here. Since a membrane can be considered as a slab of material with a finite thickness and parallel faces, the membrane problem becomes one of linear transfer. In this instance by regarding Z of equations 9 and 10 as ΔX, the thickness of the membrane in cm., which is generally unknown, we can write equation 11 as

$$-J_i = \sum_k M_{ik}\Delta\mu_k \tag{14}$$

We take equation 14 to be the general equation applying to material transfers through membranes. $\Delta\mu_k$ is the finite difference in chemical potential of a substance on the two sides of the membrane.

We may in the first instance regard the concept of force as used in equations 1 to 7 as being "unreal," but on further reflection we realize that the force used there is just as "unreal" as any of the forces used in physics. We propose that forces derived from gradients of chemical potential be called Gibbs' forces and that they take their rightful place among the forces considered in physics.

We are now in a position to state *why* substances move through membranes. The components in the membrane move because they are acted upon by forces derivable from gradients of chemical potential produced by causes external to the membrane. Substances move through a membrane because the substances are in a Gibbs field of force, just as ions move when in an electrical field of force or masses move when in a gravitational field of force. The laws of motion are similar in all instances. Furthermore, for a substance to pass through a membrane it must have a finite concentration in the membrane, a fact recognized long ago by Overton,[10] for example, but often forgotten in discussions of the permeability of membranes to ions or polar substances. It is possible, however, for the concentration of a substance in the membrane to be large but its conductance to be zero. The major difficulty in understanding transfers across membranes has been the attempt to obtain transfer equations without an adequate consideration of the forces involved or of the interactions that take place between the moving components.

Two component systems. Expanding equation 14 for a system containing water, w, and a solute, s, we write

$$\begin{aligned} -J_w &= M_{ww}\Delta\mu_w + M_{ws}\Delta\mu_s \\ -J_s &= M_{sw}\Delta\mu_w + M_{ss}\mu\Delta_s \end{aligned} \qquad (15)$$

where M_{sw} is equal to M_{ws} because of the Onsager relations. We see that three independent coefficients, M_{ww}, M_{ws}, and M_{ss}, are necessary for the phenomenological description of the system. This conclusion makes necessary a complete re-examination of all the data obtained in the study of membrane transfers, for in the case of two component systems most of the previous treatments of the transfer process have been attempted by the use of only two independent coefficients.

Unfortunately, our knowledge of the structure of particular membranes or of the intrinsic mechanisms of transfer is so incomplete that the values of the M's appropriate to a given membrane cannot be calculated except in certain idealized situations. The M's are best regarded as empirical coefficients to be determined by suitable experiments. We must remember, of course, that the magnitudes of the M's contain information as to both membrane structure and intrinsic processes of transfer.

If for all values of $\Delta\mu_w$ and $\Delta\mu_s$ we find that J_s is 0, we call the membrane a semipermeable membrane. Under these conditions M_{ws} and M_{ss} must both be equal to 0, and equation 15 becomes

$$-J_w = M_{ww}\mu\Delta_w \qquad (16)$$

In isothermal systems in which the gradients are pressure gradients and/or concentration gradients $\Delta\mu_w$ is given by

$$\Delta\mu_w = (d\mu_w/dP)\,\Delta P + (d\mu_w/d\pi_w)\Delta\pi_w = \bar{v}_w\,(\Delta P - \Delta\pi_w) \qquad (17)$$

since the slight compressibilities of the solutions are of second order. This allows us to write equation 16 as

$$-J_w = M_{ww}v_w\,(\Delta P - \Delta_w) \qquad (18)$$

We immediately recognize equation 18 as the mathematical statement of Starling's law.[18] Starling's law as applied to transfers of water between the blood and the interstitial fluids through the capillary walls is mechanically and thermodynamically sound if blood is thought of as a two-component system composed of a permeating solvent, w, and a nonpermeating solute, s. Equation 18 will hold regardless of whether we consider the blood capillary membranes as "pore" membranes or "diffusion" membranes. Note that when the same transfer coefficient is applicable to a ΔP and a $\Delta\pi_w$ as in equation 18, the membrane is a semipermeable membrane.

Equation 18 is also the basic equation for the determination of the osmotic pressure of water in a solution. When a ΔP is allowed to develop or is imposed upon the system by external means, and in consequence J_w becomes 0 then ΔP is equal to $\Delta\pi_w$. Very few membranes can be found that are ideally impermeable. In most instances the complete form of equation 15 must be used to describe the transfers of the two components. If a membrane could be found that is impermeable to the solvent but permeable to the solute, equation 18 would now hold for the solute if we replace the w subscripts with s subscripts. This is mentioned to illustrate one very important point. A solution has as many π's as there are components in the solution. Since a solute is usually regarded as the component present in small concentration, its π is large in magnitude and the movement of the solute in such a membrane system would be practically independent of the experimentally realizable pressure gradients.

Stavermann[14] has discussed the problem of osmotic measurements with permeable membranes, and has outlined the information obtainable from osmotic experiments with such membranes. Kedem and Katshalsky[6] have analyzed the situation existing in transfers of water in biological two-component systems, and have introduced a very useful classification of membranes based upon three experimentally determinable coefficients.

Fick's law. The relation of our phenomenological equations to Fick's law is easily found. In a two-component system in which the only gradient existing is a concentration gradient, and the solutions are so dilute that $\Delta\mu_w$ may be approximated by the relation

$$\Delta\mu_w = RT\,\Delta\,\ln C_w = RT\,\Delta\,C_w/C_w \qquad (17a)$$

then equation 8 can be written

$$dn_w/dt = -\,ART\lambda_{ww}\Delta\,C_w \qquad (18a)$$

We now set $RT\,\lambda_{ww}$ equal to h, the permeation coefficient of the membrane for water and we have

$$dn_w/dt = -\,Ah\,\Delta\,C_w \qquad (19)$$

which is Fick's law for a semipermeable membrane. The limited conditions under which Fick's law is applicable are not those usually found in biological systems.

Membranes and tagged components. Consider a semipermeable membrane permeable to water. If two species of water are present such as might obtain if the two species contain different isotopes, we would write for the transfer equations

$$
\begin{aligned}
-J_1 &= M_{11}\Delta\mu_1 + M_{12}\Delta\mu_2 \\
-J_2 &= M_{12}\Delta\mu_1 + M_{22}\Delta\mu_2
\end{aligned}
\tag{20}
$$

We see that three independent coefficients are again necessary for the description of the flows, since two species having different physical properties are to be regarded as independent components. If we were to impose a pressure gradient across the membrane in such a system, a partial separation of the species could be achieved because of the different flows of the two species through the membrane. Equations 20 can be taken as the basic equations describing isotope separation processes at membranes. When we employ tagged species in the study of biological processes it is customary to neglect the small effects that arise because of isotopic differences. They are usually of second order, so this is entirely proper. However, to understand the behavior of the tagged components in the system, we must write our mathematical equations as if the two species are not independent components, so far as their motions are concerned.

We can develop the appropriate equations as follows: We shall consider the two species to be identical in all respects except for one property by which we can recognize the species but which property has no effect on the resulting motions. We can say then that the membrane does not distinguish between the two species. In an actual experiment with water, oxygen-18 water would be the tagged water of choice as isotope separation effects during transfer through the membrane would be minimal, particularly if the oxygen-18 water were present in tracer concentrations.

We can visualize three possible mutually interfering flows through the membrane. A flow that takes place without a change in the molar ratio of the two species and the two flows of the individual species which lead to changes in the molar ratios. Our formal phenomenological relations for these three flows would be

$$
\begin{aligned}
-J_w &= M_{ww}\Delta\mu_w + M_{w1}\Delta\mu_1 + M_{w2}\Delta\mu_2 \\
-J_1 &= M_{w1}\Delta\mu_w + M_{11}\Delta\mu_1 + M_{12}\Delta\mu_2 \\
-J_2 &= M_{w2}\Delta\mu_w + M_{12}\Delta\mu_1 + M_{22}\Delta\mu_2
\end{aligned}
\tag{21}
$$

where all the M's are positive. The $\Delta\mu$'s of equations 21 are not independent variables. They can be expressed terms of $\Delta\mu_w$ and ΔN_1 or ΔN_2 where N_1 and N_2 are the *species mol fractions*. We define the species mol fractions and the relations between them as follows

$$
\begin{aligned}
&n_1 + n_2 = n_w\,, \quad N_1 = n_1/n_w \quad N_2 = n_2/n_w \\
&N_1 + N_2 = 1 \quad \Delta N_1 = -\Delta N_2
\end{aligned}
\tag{22}
$$

If we disregard for the moment the two species of water, we may write for $\Delta\mu_w$ that

$$\Delta\mu_w = RT\ \Delta\ \ln p_w \tag{23}$$

where p_w can be called the fugacity or the escaping tendency of water. This we take as proportional to the vapor pressure of water. The vapor pressure of the species "i" would be given by $N_i p_w$, so that we may write

$$\begin{aligned}\Delta\mu_1 &= RT\Delta\ln N_1 p_w = \Delta\mu_w + RT\Delta\ln N_1 \\ \Delta\mu_2 &= RT\Delta\ln N_2 p_w = \Delta\mu_w + RT\Delta\ln N_2\end{aligned} \tag{24}$$

If ΔN_2 is small in comparison to N_1 and N_2, then we can write equations 24 as

$$\begin{aligned}\Delta\mu_1 &= \Delta\mu_w - RT\ \Delta N_2/N_1 \\ \Delta\mu_2 &= \Delta\mu_w + RT\ \Delta N_2/N_2\end{aligned} \tag{25}$$

By substitution of the results into equation 20 we obtain

$$\begin{aligned}-J_w &= (M_{ww} + M_{w1} + M_{w2})\Delta\mu_w + \left(\frac{M_{w2}}{N_2} - \frac{M_{w1}}{N_1}\right)RT\Delta N_2 \\ -J_1 &= (M_{w1} + M_{11} + M_{12})\Delta\mu_w + \left(\frac{M_{12}}{N_2} - \frac{M_{11}}{N_1}\right)RT\Delta N_2 \\ -J_2 &= (M_{w2} + M_{12} + M_{22})\Delta\mu_w + \left(\frac{M_{22}}{N_2} - \frac{M_{12}}{N_1}\right)RT\Delta N_2\end{aligned} \tag{26}$$

which hold for all values of $\Delta\mu_w$ and ΔN_2. When $\Delta\mu_w$ is 0, J_w must be 0 since a ΔN_2 cannot produce a flow of constant composition. This requires that

$$M_{w2}/N_2 = M_{w1}/N_1 \tag{27}$$

Also when $\Delta\mu_w$ is 0, J_1 plus J_2 is also 0, an exchange process of identical material between the two compartments of the system is the only process taking place. This requires that

$$\frac{M_{11}}{N_1} - \frac{M_{12}}{N_2} = \frac{M_{22}}{N_2} - \frac{M_{12}}{N_1} = M_e \tag{28}$$

We can call M_e the exchange coefficient. It is also a positive quantity and therefore M_{11}/N_1 is greater than M_{12}/N_2 and M_{22}/N_2 is greater than M_{12}/N_1. The fluxes of equations 26 are not directly observable in the

general situation. The following quantities, indicated by the superscript *, are experimentally determinable

$$\begin{aligned} J_w^* &= -M_{ww}^* \Delta\mu_w = J_w + J_1 + J_2 \\ J_1^* &= N_1 J_w + J_1 \\ J_2^* &= N_2 J_w + J_2 \end{aligned} \tag{29}$$

From equations 26, 27, 28, and 29, after the necessary algebraic manipulation, we obtain

$$\begin{aligned} J_1^* &= -N_1 M_{ww}^* \Delta\mu_w + M_e RT\Delta N_2 \\ J_2^* &= -N_2 M_{ww}^* \Delta\mu_w - M_e RT\Delta N_2 \end{aligned} \tag{30}$$

in which

$$M_{ww}^* = M_{ww} + \frac{2M_{w1} + M_{11} + M_{12}}{N_1} \tag{31}$$

From equations 29 and 30 we see that in order to describe the processes occurring in a semipermeable membrane system containing two species of water, two independent coefficients are necessary, the osmotic coefficient, M_{ww}^* and the exchange coefficient M_e. These coefficients are best determined in separate experiments. In the first experiment we could arrange for pressure gradients to be absent and determine the quantity of water that flows through the membrane in a unit time. If the nonpermeable material in the system is present at low concentration then

$$dn_w/dt = -A(M_{ww}^*/N_w)RT\Delta N_w \tag{32}$$

an equation which is Fick's law when the concentration is expressed in mol fractions. In the above experiment N_w can be taken as unity, since pure water can always be placed in one of the compartments and the outflow of water from this compartment can be determined. We would arrange the second experiment so that J_w is 0, but have ΔN_2 finite. The results of the second experiment would be represented by

$$dn_2/dt = -AM_e RT\Delta N_2 \tag{33}$$

The ratio of the osmotic coefficient to the exchange coefficient is always greater than one, for

$$M_{ww}^*/M_e = \frac{M_{ww} + \dfrac{2M_{w1} + M_{11} + M_{12}}{N_1}}{\dfrac{M_{11}}{N_1} - \dfrac{M_{12}}{N_2}} > 1 \tag{34}$$

since

$$\frac{M_{11}}{N_1} > \frac{M_{11}}{N_1} - \frac{M_{12}}{N_2} \tag{35}$$

The experiments performed on a frog by Hevesy, Hofer, and Krogh[6] were essentially of this type, and we see that we need not be surprised that two coefficients were needed in the expression of their results, even though they used equations of the form of Fick's law to evaluate their findings. They found a ratio of M^*_{ww}/M_e for their system to be about 5. The physical significance of this consequence is readily apparent. In the osmotic experiment the resistance to the flow of water is the resistance of the membrane. In the exchange experiment the resistance to the flow of species one is the resistance of the membrane plus the resistance generated by the counter current flow of the second species.

Tagged species in multicomponent systems. We can easily obtain the equations for two species of one component in a multicomponent system by methods similar to those outlined above. The equations for component "i" where "i" can be either the solvent or one of the solutes are

$$\begin{aligned} J^*_1 &= N_1 J^*_i + M_e RT \Delta N_2 \\ J^*_2 &= N_2 J^*_i - M_e RT \Delta N_2 \end{aligned} \tag{36}$$

If we let subscript 2 refer to the tagged species present in "tracer" amounts, we see that J^*_2 is practically independent of changes in the flux of the normal solvent or solute respectively. In fact, J^* is practically independent of all other gradients, including pressure gradients. This behavior of the solvent was observed in the experiments reported by Hevesy, Hofer, and Krogh,[6] Ussing,[16] or Durbin, Frank, and Solomon.[3] Similar experiments on tagged solutes do not seem to have been carried out.

Equations 36 are particularly instructive, for they render invalid one of the assumptions often made in the application of tagged materials to the study of the properties of membranes. The assumption often made is that the flow of the tagged material is proportional to the flow of normal material. This is true only when ΔN_2 is 0, or the molar ratio of the two species is the same in all the compartments of a multicompartment system. The addition of a tagged species to one compartment of the system in tracer amounts cannot give any information as to the flows of the normal components, for these flows depend upon independent undetermined coefficients other than M_e. In this sense a "tracer" does not trace the flow of the normal component.

The present theory of "tracer" applications to the kinetic behavior of biological systems has been outlined by Robertson.[11] It is derived from the assumption that effluxes and influxes of a tagged species from a particular compartment are obtained by multiplying the efflux or influx of a component by the species mol fraction of the component from the compartment from which the flow originates. This assumption is easily shown to be correct in chemical reaction systems if one assumes that the species have identical chemical reaction velocity constants. This assumption is invalid, however, for transfer processes at membranes, for the observable transfer of the component is dependent upon osmotic coefficients, while the observable transfer of the isotope species is dependent upon both the osmotic coefficients and the exchange coefficient. The assumption has been questioned before, particularly by Edwards and Harris,[3] and they give a model for a membrane to demonstrate how the nonvalidity of the assumption might be accounted for. Sheppard,[12] in addition, has previously obtained an equation of the form of equation 36 which unfortunately was never published. Therefore, while the addition of tagged components to one compartment of a system has proven that biological membranes hitherto considered impermeable to certain substances are actually permeable, the results of such tracer experiments alone can only set a minimal value for the transfer rate of the normal component across the membrane. The actual rate may be many times this minimal rate.

Model theory. Our phenomenological equations are valid irrespective of the mechanisms of transfer or structure of the membrane. If, however, we were to assume that the membrane contains pores of a radius large enough that Poiseuille's law for stream line flow is valid, then M^*_{ww} becomes a function of Poiseuille's coefficient, and M_e is related to the self diffusion coefficient of water. The magnitude of the ratio M^*_{ww}/M_e becomes of the order of $r^2 \times 10^{15}$ when r is expressed in centimeters. For large pores such as blood vessels or pipe lines the region of diffusion exchange would travel with the stream velocity. Under these conditions the flux of one species is proportional to the flux of the second species across a plane in the pore. This is the application made of isotopes to determine blood velocity in the arteries or the flow of a given quantity of material in pipe lines.

The situation obtaining as pore radius approaches molecular dimensions becomes exceedingly difficult both conceptually and mathematically. If we choose to consider that Poiseuille's law is valid in this region, the "effective" pore sizes calculated from any experimentally determined values of transfer are not likely to bear any simple relation to physical reality. We are most likely using an hydraulic analogue as an heuristic device for the description of the transfer process.

By using properties other than those directly determinable by transfer experiments we might give an experimental definition of a pore membrane. From a naïve point of view we expect a pore membrane to have stable dimensions when subjected to cycles of hydration and dehydration. Thus a sintered glass disk or a frit is a pore membrane by this definition. During hydration and dehydration water merely displaces or is displaced by air. In contrast, many of the membranes of biological interest are not dimensionally stable to hydration and dehydration. The dry membranes often swell when placed in water and the membrane may be thought of as a colloidal structure. Transfers through such structures would be in the nature of random processes, that is, modified diffusion processes. The criterion of whether a substance penetrates a membrane becomes simply the experimentally ascertainable fact. Is the substance soluble in the membrane?

If the membrane is permeable to k different components and k components are present in the system, then a maximum of $1/2\ k\ (k + 1)$ independent M's may be necessary for the description of the system. When we recognize this fact, we then appreciate how little we know concerning the transfer relations at any biological membrane. We must accept the fact that the transfer of any component may affect and be affected by all the other components in the system. The task ahead is the determination of the admittance matrices for those limiting boundaries which separate living cells from their external environments and seemingly make life possible.

CONCLUSIONS

1. The theory of coupled steady state processes provides a powerful tool for the quantitative study of transfers in biological systems.

2. When the theory is applied to typical applications of tagged materials to the study of transfers through membranes, an explanation is afforded for experimental results whcih have been difficult to reconcile by incomplete theories.

3. A tagged material "traces" the flow of the untagged substance through a membrane when and only when the molar ratio of the tagged to untagged substance is the same in all compartments.

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AIRWAY RESISTANCE AND LUNG COMPLIANCE. The effects of histamine and hyperventilation in normal subjects and patients with chronic lung disease¶

Lung compliance may be defined as the volume change per unit pressure change.[a] It is an expression of the elastic-like properties of the lung if it is measured at points on a pressure-volume curve at which air is not moving into or out of the lung. These points of no air-flow occur near the end of inspiration and again near the end of expiration. Compliance does not include air resistive changes which are the results of friction between the airstream and bronchial wall, turbulance, and other aerodynamic factors. However, studies of lung compliance should yield information about changes in pulmonary mechanics which, although separate from airway resistance, may be influenced by it. The present study represents an attempt deliberately to alter airway resistance by using a known bronchoconstrictor (histamine) in normal subjects and in patients with chronic lung diseases. The influence of this bronchoconstriction on compliance was observed and compared to the effect of hyperventilation.

MATERIAL AND METHODS

Seventeen normal individuals, 9 men and 8 women, ranging in age from 19 to 48 years, and 17 patients, 12 men and 5 women varying in age from 25 to 74 years with various lung diseases, were studied (Tables 1 and 2). Estimations of lung compliance and airway resistance were carried out in the sitting position at rest and during hyperventilation. All subjects were then given 0.5 to 0.7 mgm. of histamine phosphate subcutaneously, and estimations of compliance and resistance again carried out at rest and during hyperventilation after 4, 8, and 12 minutes. The observations made 8 minutes

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¶ This investigation was supported by a research grant (H-1516 C3) from the National Heart Institute, U. S. Public Health Service.
Received for publication March 13, 1959.

after histamine have been used because the change from the base-line values were most pronounced at that time. Two of the patients became severely dyspneic after histamine and, in one case (W.G.), the study was ended after 4 minutes. These patients improved promptly after receiving 0.3 mgm. of epinephrine hydrochloride subcutaneously.

Intra-esophageal pressure was measured by the balloon technique,[6] the balloon being introduced through the mouth after anesthetizing the pharynx with benadryl, 0.25 per cent. The polyethylene catheter to which the balloon was attached was connected to one side of a Sanborn differential pressure transducer, and a polyethylene tube leading from the mouth-piece was connected to the other side of the transducer, thus differential mouth-intraesophageal pressure could be recorded on a direct-writing oscillograph. The subject breathed through a pneumotachograph; tidal volume and flow rate were recorded on the Sanborn apparatus through a Lilly manometer and integrator.

In this way three curves were recorded simultaneously: differential mouth-esophageal pressure, tidal volume, and respiratory flow rate. The effective compliance was calculated, measuring tidal volume and esophageal pressure at corresponding points of no airflow, as seen from the flow rate curve. In this paper the term "compliance" will refer to the volume to pressure change relationship at points of no air flow. It is expressed by the formula:

$$\text{Compliance} = \frac{\text{Volume change in liters}}{\text{Pressure change in cm. } H_2O}$$

The same three curves were used to calculate airway resistance.[4] Points on the ascending and descending limbs of the tidal volume curve were chosen which represented points of equal inflation and deflation. Vertical lines from these points transected the pressure and velocity curves, and the difference of pressures and velocities between the two points provided the data for the formula:

$$\text{Airway Resistance} = \frac{\text{Pressure difference in cm. } H_2O}{\text{Velocity difference in L./sec.}}$$

RESULTS

The lung compliance in the normal individuals varied from 0.109 to 0.328 L/cm. H_20 (Table 1). The lowest value was found in a small woman and the highest value in a large woman. This was expected, since previous studies have shown a relationship between lung compliance and body size in normal subjects.[2,7] The diagnosis in each of the patients was based on a careful review of the history, physical findings, and chest x-ray coupled with compatible pulmonary function tests (lung volumes, maximal breathing capacity, intrapulmonary gas distribution, and oximetry).

Clinically the patients fell into two groups: one with a diagnosis of pulmonary emphysema; the other with a diagnosis of pulmonary fibrosis or granulomatosis. The average resting compliance was lower, and air flow resistance higher than normal in both groups of patients.

When resistance was low, compliance varied greatly, but as resistance increased, compliance tended to be low (Fig. 1). The high resistances were

TABLE 1. DATA FROM NORMAL SUBJECTS

Subject	Sex	Body surface area, sq. M.	Before histamine: Compliance L/cm H_2O Rest	Before histamine: Compliance L/cm H_2O Hypervent.	Before histamine: F	Before histamine: Resistance cm H_2O/L/sec. Rest	Before histamine: Resistance cm H_2O/L/sec. Hypervent.	After histamine: Compliance L/cm H_2O Rest	After histamine: Compliance L/cm H_2O Hypervent.	After histamine: F	After histamine: Resistance cm H_2O/L/sec. Rest	After histamine: Resistance cm H_2O/L/sec. Hypervent.
Part A—Resistance Increased												
A.F.	M	2.05	0.287	0.329	60	1.31	2.55	0.185	0.187	50	3.20	1.56
O.S.	M	2.05	0.204	0.162	60	1.42	5.60	0.120	0.150	80	7.70	8.55
E.F.	M	1.94	0.223	0.128	85	1.81	1.98	0.191	0.134	130	2.73	3.53
F.F.	M	1.78	0.166	0.117	90	0.94	1.31	0.166	0.166	75	3.58	...
D.S.	M	1.86	0.212	0.139	100	0.67	2.66	0.192	0.204	100	2.62	3.15
L.S.	F	1.79	0.187	0.174	105	1.00	3.82	0.173	0.169	120	1.23	4.45
S.R.	F	1.74	0.190	0.194	60	0.73	1.16	0.241	0.256	60	1.28	2.46
R.S.	F	1.74	0.191	0.225	65	1.03	1.43	0.183	0.073	60	1.19	2.23
S.L.	F	1.76	0.194	0.158	80	1.55	2.78	0.174	0.176	60	1.69	3.62
L.L.	F	1.48	0.109	0.130	90	1.74	1.87	0.151	0.079	100	6.45	7.42
E.H.	F	1.84	0.211	0.140	90	1.42	1.53	0.158	0.152	60	2.00	...
Part B—Resistance Decreased												
L.C.	M	1.80	0.116	0.074	60	2.10	...	0.126	0.113	60	1.90	2.72
R.M.	M	1.79	0.162	0.133	60	3.37	3.92	0.174	0.220	55	2.10	3.21
L.P.	M	1.84	0.284	0.128	105	...	4.10	0.258	0.177	105	...	3.50
D.K.	F	1.86	0.267	0.257	70	1.65	1.22	0.231	0.173	70	1.24	1.96
A.A.*	F	1.72	0.125	0.155	60	5.17	6.44	0.158	0.181	60	3.70	5.45
E.L.	M	1.88	0.328	0.213	60	1.37	1.33	0.260	0.231	60	0.82	1.47

* No explanation could be found for these values in an otherwise normal subject.
F = Respiratory rate.

mainly but not entirely found in the patient group. Histamine generally produced an increase in resistance, but in 6 of 17 normal subjects and 6 of 17 patients this did not occur (Tables 1 and 2). After histamine the resistance increased in all but one of the 11 patients with emphysema and in only one of the 6 patients with fibrosis. Histamine, hyperventilation, and both together tended to decrease compliance as resistance increased

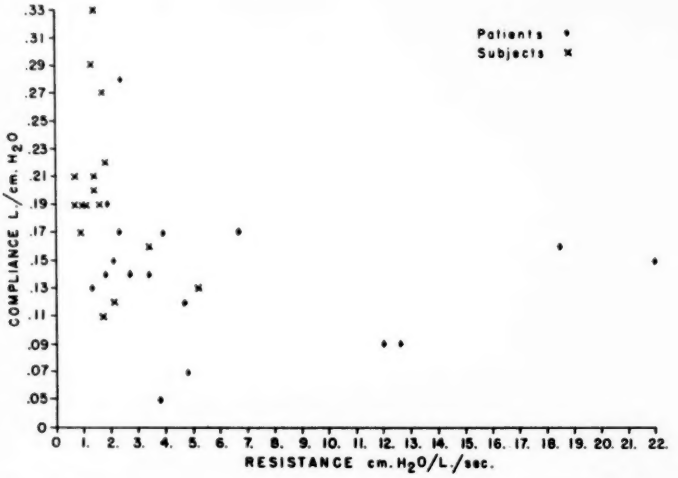

FIG. 1. Relationship between resistance and compliance. Patients and normal subjects.

(Fig. 2). The effect of histamine on compliance was quite variable in patients and normal subjects, but the usual effect was to increase resistance and concomitantly reduce compliance (Fig. 2). Hyperventilation generally increased resistance and decreased compliance both before and after histamine.

Figure 3 shows the effect of hyperventilation at different levels of lung inflation in a normal subject. The greatest fall in compliance with hyperventilation in this individual occurred when the end expiratory level was established at maximal expiration, the least fall when the expiratory level was about 30 per cent above the normal resting mid-position. Airflow resistance was high at either extreme of mid-position when the respiratory (and flow) rate was high. At moderate rates airway resistance changed very little.

TABLE 2. PATIENT DATA

Pt.	Sex	Body surface area, sq. M	Before histamine: Compliance L/cm H_2O Rest	Before histamine: Compliance L/cm H_2O Hypervent.	F	Before histamine: Resistance cm H_2O/L/sec. Rest	Before histamine: Resistance cm H_2O/L/sec. Hypervent.	After histamine: Compliance L/cm H_2O Rest	After histamine: Compliance L/cm H_2O Hypervent.	F	After histamine: Resistance cm H_2O/L/sec. Rest	After histamine: Resistance cm H_2O/L/sec. Hypervent.	Diag.
Part A—Resistance increased													
H.P.	M	1.88	0.088	0.023	65	12.00	12.60	0.043	....	...	22.00	....	P.F.
J.M.	M	1.76	0.167	0.133	35	3.85	10.70	0.081	0.032	60	15.80	15.60	P.E.
V.B.	F	1.70	0.121	0.098	75	1.83	1.29	0.089	0.103	70	2.38	1.62	P.E.
H.B.	M	1.48	0.190	....	...	1.94	....	0.110	....	...	3.10	....	P.E.
J.M.	M	1.80	0.162	0.059	72	18.54	22.40	0.113	0.015	72	40.00	28.28	P.E.
N.P.	M	1.94	0.142	0.142	25	3.43	4.37	0.068	0.032	90	9.50	....	P.E.
J.B.	M	1.84	0.128	0.063	40	1.34	4.70	0.079	0.052	30	6.90	....	P.E.
B.J.	M	1.86	0.124	0.057	108	4.73	5.97	0.104	0.040	85	9.65	10.55	P.E.
W.G.*	M	2.00	0.151	0.058	70	22.00	20.40	0.096	0.052	60	24.40	....	P.E.
F.T.	M	1.88	0.169	0.133	120	6.66	7.99	0.135	0.095	160	15.30	....	P.E.
E.P.	F	1.68	0.148	0.136	100	2.08	5.47	0.123	0.083	100	4.31	5.45	P.E.
Part B—Resistance decreased													
P.M.	M	1.70	0.054	0.038	100	3.79	2.70	0.048	0.035	150	3.25	2.56	P.F.
F.C.	F	1.64	0.088	0.034	70	12.60	11.29	0.071	0.042	60	12.36	15.05	P.F.
M.W.	F	1.76	0.068	0.059	110	4.80	4.56	0.074	0.068	135	2.60	4.70	P.F.
L.H.	M	2.04	0.275	0.163	115	2.42	4.37	0.307	0.175	120	2.20	4.50	P.F.
H.B.	F	1.70	0.136	0.143	120	2.65	3.87	0.148	0.084	165	2.27	4.44	P.F.
L.T.	M	1.88	0.168	0.100	140	2.47	3.90	0.164	0.071	120	2.35	4.30	P.E.

* This study was ended after 4 minutes because of severe dyspnea.
F = Respiratory rate.
PE = Pulmonary emphysema.
PF = Pulmonary fibrosis.

DISCUSSION

These studies have shown that factors which tend to increase resistance above the range of normal values are associated with a decrease in compliance. Why compliance changes in this way is not clear, and, at, present,

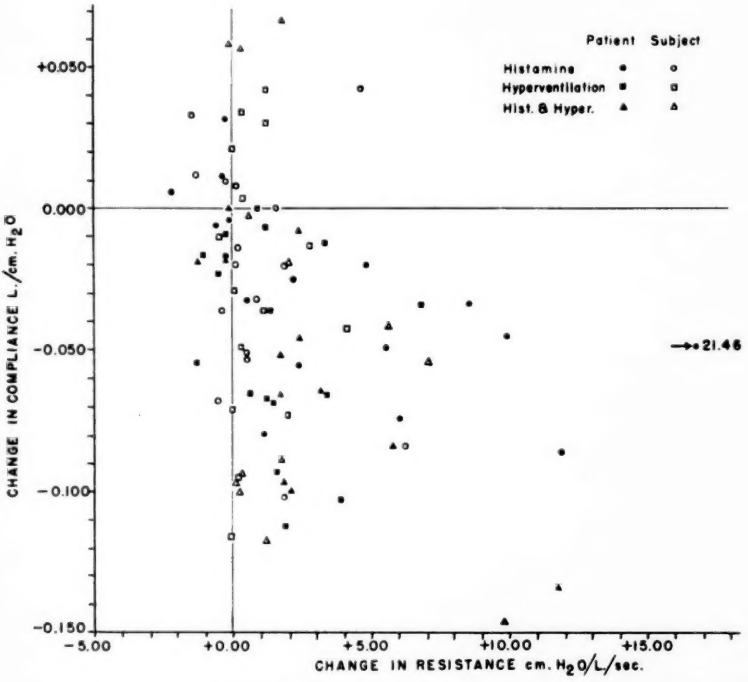

FIG. 2. Relationship between changes in resistance and concomitant changes in compliance. Patients and normal subjects, effects of histamine, and hyperventilation.

only subject to speculation. In our analytical methods we have treated a nonuniform mechanical system as though it were uniform. Clearly we cannot expect such methods to do more than suggest the underlying characteristics.

The sizable proportion of both patients and normal subjects who showed no decrease in resistance after histamine suggests individual dose variation. The dose was small, and it was anticipated that some of the patients would not react. Others reacted violently, hence it was not prudent to use

a larger dose. The singular tendency of the patients to arrange themselves into an emphysema group in which resistance increased and a fibrosis group in which it did not suggests that the patients with emphysema in this study may have had more sensitive bronchial musculature.

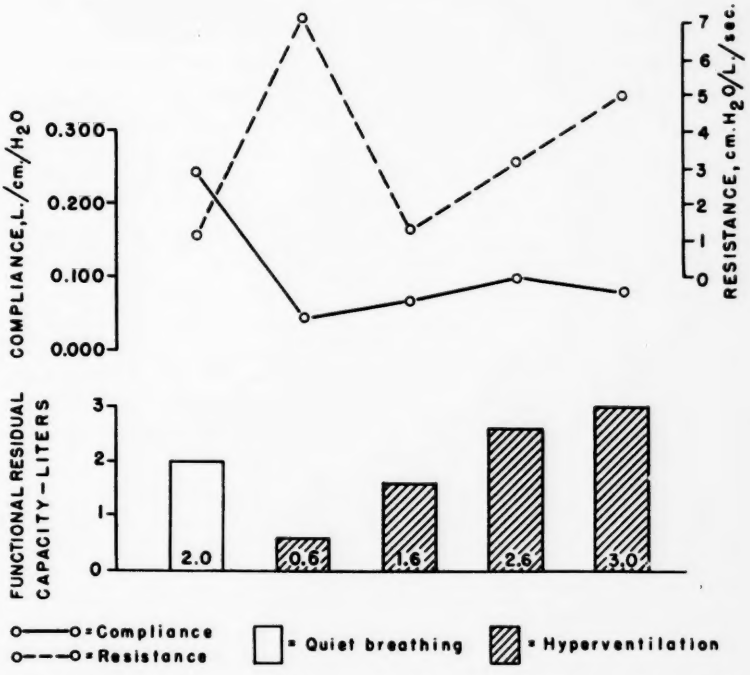

FIG. 3. Effects of hyperventilation on resistance and compliance at varying mid-positions. (FRC was determined by standard technique during quiet breathing through a pneumotachograph with an integrator for tidal volume. As the subject raised or lowered his mid-position, corresponding amounts were added to or subtracted from the initial FRC).

The attempt to produce an increase in resistance by the use of histamine was successful in only some of the normal subjects and patients, and generally not as effective as hyperventilation. Hyperventilation probably increases airway resistance by virtue of forming eddy flow or turbulent flow. The high intrapleural pressure initiating expiration during hyperventilation would tend to collapse the smaller bronchi whose lumens, very transiently, have a lower pressure. Finally, high velocity breathing might alter the

surface tension or thickness of the layer of mucous secretion on the surface of the bronchial epithelium.

Hyperventilation usually results in a greater degree of lung expansion and may raise the resting level of the lung. Figure 3 shows that compliance tends to fall off at a very high resting position. However, the drop in compliance may reflect the rapidity of the chest bellows action rather than the mid-position, since the respiratory rate is higher during hyperventilation. Thus, the sticking together and pulling apart of alveolar walls could become more of a factor at high rates of breathing.

The decrease in compliance seen in the normal individuals during hyperventilation is at variance with the findings of Cherniack[2] and Otis, *et al.*[9] These workers found no change in compliance in normal individuals during hyperventilation, a finding which they interpreted as evidence of a normal distribution of ventilation. An explanation of the difference might be that our normal individuals performed their hyperventilation in an overinflated state in which the lung elasticity had moved outside the straight part of the pressure volume curve.[9] However, in a study of normal subjects this was not supported (Fig. 3), since in some cases the compliance was actually lower when hyperventilation was carried out from the resting (mid-position) than when it was done in the inspired position. The part played by air inertia at high respiratory rates is uncertain, but could contribute to excessive pressure swings leading to low static compliance.

Although increased resistance due to histamine, when it occurred, had a depressing effect on compliance similar to that of hyperventilation, there is no reason to believe that the mechanism is the same.

The decreased compliance following histamine in those subjects and patients who had an increased airflow resistance may have represented a histamine effect on the pulmonary vasculature, but more likely it was related to the broncho-constricting effect of histamine. The drop in compliance was most notable in the patients with emphysema. This has been noted by others.[5] A possible explanation is that in the case of the emphysematous lung, because of variable distribution of ventilation, one is dealing with a lung having a family of compliance curves rather than a single curve. Under such circumstances one may not be measuring compliance at a point of "no flow," since continuous movement of air in some parts of the lung may still be present after air flow at the mouth has ceased. Such patterns of uneven segmental ventilation could give apparent changes in static compliance. One factor in ventilation known to be affected by histamine is the collateral respiration which is diminished by histamine in dogs.[3] Very little is known about the effect of collateral respiration on lung compliance.

Whatever may be the reason that histamine reduces compliance in normal subjects and patients with emphysema does not apply to patients with pulmonary fibrosis. There was no consistent drop in compliance after histamine in the latter group (Table 2). These patients had a low compliance to begin with. In fact, histamine had no demonstrable effect on their ventilatory mechanics, and one might infer that they tolerate histamine unusually well. They did experience tachycardia, skin flush, and headache.

The drop in compliance and its resulting dyspnea after histamine in patients with emphysema resembles the difficulty experienced by these individuals after even a mild exacerbation of chronic bronchitis or an upper respiratory tract infection.

SUMMARY

1. Lung compliance and airway resistance were measured in 17 normal subjects and 17 patients with various types of chronic lung disease both at rest and during hyperventilation before and after the injection of histamine.

2. Compliance was in the low normal range for the patients with emphysema, but definitely low for patients with pulmonary fibrosis. Histamine and hyperventilation further lowered the compliance in the patients with emphysema, but had a less pronounced effect on the patients with fibrosis and in normal subjects.

3. When airway resistance was low, compliance varied greatly, but when resistance was high, compliance tended to be low.

4. Airway resistance was initially high in most of the patients. It increased following histamine injection and hyperventilation in most of the normal subjects and patients. Hyperventilation had a more pronounced effect than histamine.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the help of Dr. Colin White and his staff of the Department of Public Health in preparing the presentation of the data, and to the Misses Adrienne Audette, Susan Miranda, and Liv Storstein for their technical assistance.

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THE STRUGGLE AGAINST SEPSIS**

The struggle against sepsis is as old as man. Primitive man believed that infections were inflicted upon him by the gods or by evil spirits. The Bible reflects this belief in that the boils of the plagues of Egypt were brought about under the Lord's direction, and Job's boils were due to Satan's ministrations.[15]

The Hippocratic School made the first attempt to deal with the surgical treatment of infection. Cleanliness and little interference with natural processes resulted in prompt healing of incised wounds.[1] Infected wounds and empyema were well handled.[95]

From the time of Hippocrates down to Pasteur's brilliant studies on fermentation, there were two schools of thought regarding the treatment of wounds. The Hippocratic doctrine of letting Nature take the lead in healing was forgotten and rediscovered many times. Galen, though he made many contributions to medicine, introduced the idea that suppuration was essential to the healing of wounds.[58] Followers of Galen introduced salves, and plasters into wounds.[2, 4, 59, 122]

The Muslims extolled the virtues of fire over the cold knife in surgery and this doctrine held well into the 15th century.[5, 6, 59] During the Middle Ages, Galen's philosophy of the necessity for pus formation for healing was forwarded by Roger of Palermo's introduction in 1170 of the seton—a bundle of linen threads stitched through a fold of skin and led to the exterior.[130] Guy de Chauliac supported Galen's idea of suppuration as essential to healing.[67] Three other surgeons had the courage to deny that suppuration was necessary to healing though they made little impression on their colleagues.[108, 186, 152]

War wounds were thought by both Brunschwig and Vigo to be poisoned.[19, 169] This led to disagreement among some surgeons.[80, 118] Paré followed Vigo in practice although later he fully abandoned the boiling oil treatment of war wounds. His theory of wound treatment as well as

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** The Fourth Samuel Clark Harvey Lecture read Monday, 9 March 1959, in the Historical Library, Yale University School of Medicine.

his other contributions are well known to all students of medical history[115, 119, 120]

Botallo investigated gunpowder experimentally and found that there was no poison principle involved. He believed that foreign bodies in the wounds such as bone fragments, contused and lacerated tissues, blood clots, and metallic objects were responsible for the suppuration.[18] Vicary agreed that removal of these objects was necessary for good healing.[125, 126] Della Croce[30] warned against leaving dead spaces and advocated clean handling of wounds.

Speed in surgery was an essential before the discovery of anesthesia. Cheselden[65] was credited with removal of a bladder stone in an operation which required 54 seconds. John Hunter, his pupil, advanced surgery to the status of a true science wherein it was necessary for the surgeon to consider the physiology and pathology as well as the surgical manipulations. His studies on the blood, inflammation, and gunshot wounds were classic.[75, 82, 110, 116, 117] He showed that an incised wound healed by adhesive inflammation. That putrefaction was still expected in a large proportion of operations at that time can be seen in the following entry from Astley Cooper (1768-1841) who removed a sebaceous cyst from the scalp of George IV and told how he was terrified lest infection should occur. Cooper wrote: "I felt giddy at the idea of my fate hanging on such an event. I am certain that if anything happened to the King that, at any rate, I should leave London and live in retirement."

John Bell (1763-1820) wrote that suppuration would be prevented in almost every case if the surgeon saw to "the laying of the wounded parts so cleanly, so neatly, and so evenly in contact with each other that they may adhere." The operation should be completed with a dry wound.[11]

It has been stated that between the years 1800-50 in civil practice there was a 50 per cent mortality for most operations. Hospitals had an especially bad name because they seemed to have epidemics of infection. Diseases such as pyaemia, septicemia, erysipelas, and gangrene were likely to follow any hospital operation. Tetanus and edematous gangrene also occurred and were greatly feared. These diseases were thought to be contagious, and overcrowding was considered the cause. Sir James Y. Simpson of Edinburgh was convinced that the only way to abolish this threat was to tear down all the hospitals and to erect widely separated cheap cottages in their stead. These could be torn down and burned every few years.[145]

Pirogoff wrote from the Second Continental Hospital at St. Petersburg: "The large, badly ventilated wards are filled with patients suffering from erysipelas, acute and purulent edemas and septicaemia. The nurses, with-

out any scruples, were in the habit of transferring the linen serving as compresses for the wounds from one patient to another. The mal-administration of hospitals went so far as to preserve for the purpose of resale, soiled and ill-smelling lint taken from the wounds as well as the dressings, compresses and linen, which were put up in special stockrooms situated close to the sick wards."[105, 123] Simpson found that less infection occurred in amputations in the country than when done in the city hospitals.[145] Mr. Paget in 1862 made the observation that the best results in cases of pyaemia which he had seen were in patients that had been kept day and night in a current of air. This was commented upon as being one of the great suggestions for curing the evil of blood poisoning.[88]

From John Hunter to Lister, surgery remained undeveloped because the basic underlying problems had not been solved—the control of hemorrhage partly, the control of pain poorly, and the control of infection—not at all. No two surgeons have probably ever been as skillful as were Robert Liston (1794-1847) and James Syme (1799-1870). Liston, a giant of a man could hold up the femoral vessels and accomplish a thigh amputation in 28 seconds. Syme was equally skilled and daring, opening huge aneurysms and securing the bleeding vessels inside the sac.[121] The measure of a surgeon's skill was recorded in seconds, a practice which was emulated into the 20th century by many surgeons.[65]

In spite of the discovery of bacteria by the pioneer microscopist Anthony von Leeuwenhoeck (1632-1723), their significance was not understood by scientists.[89] There existed an almost universal belief in "spontaneous generation," i.e., that changes occurred in various solutions left exposed to the air. Whoever originated the idea, it certainly appealed to the scientists of the time. Theodor Schwann upset this belief by his experiments demonstrating that there were "germs of fungi and infusoria . . . present in the air which are destroyed by glowing out the air." Putrefaction was due to these germs developing and nourishing themselves at the cost of the organic substance.[140, 153]

Berzelius, the noted Swedish chemist, Virchow, the great German pathologist, and Liebig, one of the foremost German chemists, were all opponents of the germ theory.[104, 85] Gay-Lussac showed that the air in sealed preserved bottled foods had no oxygen in it and believed that oxygen caused the fermentation.[94, 71] Liebig accepted this explanation. He claimed that alcoholic fermentation fulfilled the law of simple proportions in that $C_6H_{12}O_6$ became $2C_2H_5OH + 2CO_2$.[71]

Pasteur proved that a definite kind of fermentation was characteristic of different organisms. Yeasts produced alcohol, lactic acid bacteria formed

lactic acid, vibrion butyrique made butyric acid. He showed that fermentation in wine could be blocked by controlled heat 50° C. for a short time (Pasteurization). He even corrected Liebig's chemistry by showing that fractions of glycerine and succinic acid also appeared as well as alcohol and CO_2 in alcoholic fermentation. Pasteur's biological theory of fermentation became the cornerstone of modern medicine.[105, 102]

When Thomas Anderson, professor of chemistry at Glasgow, called the attention of Joseph Lister to Pasteur's work on fermentation, Lister realized that this was the clue to the cause of suppuration in wounds. Pasteur's demonstration as early as 1860 that spontaneous generation was due to living organisms in the air had passed unnoticed by the profession. Lister at once set out to prevent the germs in the air from reaching his operative wounds during operation. He selected carbolic acid, which had been used to purify the sewage in the nearby town of Carlisle, as the chemical to counteract the germs. He published his famous papers in 1867.[88,89] His results were striking and should have been convincing.

Lister developed absorbable catgut so that it could be cut near the knot and covered with tissues instead of hanging as long strands out of the wound. Two dangers were avoided in this way, secondary infection by extension along the suture and secondary hemorrhage from the infected large vessel. He also tried repeatedly to improve his dressings after operation. He used oakum, carbolic acid gauze, and "the antiseptic gauze," which had a thin, pink-stained piece of mackintosh between the 7th and 8th layers. This was used by him until 1889 and was still in use by the British Expeditionary Forces in 1917. Lister maintained that a dressing without antiseptic failed to prevent infection which might start from the skin near the incision. He was very careful to have dressings changed before they became soaked with discharges. Infection might come through the dressing from the bed clothing or mattress otherwise.[90]

Reaction to Lister's idea of antisepsis was immediate. He was criticized by his colleagues who claimed to have used carbolic acid before him, claimed, too, that equally good results had been achieved by others by keeping the oxygen out of the wounds, and that there was nothing new in the carbolic acid treatment. To these criticisms Lister patiently responded. He claimed no priority for the use of carbolic acid but emphasized that the method of its employment was the new principle. He warned that surgeons must not expect carbolic acid or any other agent to act like a charm unless these agents were used with strict attention to details. The results depended "on the wonderful powers of recovery possessed by

injured parts when efficiently protected against the pernicious influence of decomposition."[90, 91]

The most severe criticism came from Sir James Y. Simpson, famous for his discovery of chloroform as an anaesthetic. He claimed that all this work had been forestalled by the French, Germans, Spanish, and others. "If Mr. Lister had taken the slightest trouble to search the English medical literature alone" etc.; there followed a general dressing-down for lack of originality and plagiarism.[144] Mr. Lister replied with dignity to the editor of the *Lancet:* "As I have already endeavored to place the matter in its true light without doing injustice to anyone, I must forbear from any comment on his allegations."[92]

One should note that Mr. Simpson thought that he himself had all but banished suppuration from those hospitals which used his own method of acupressure. This was a method of arresting hemorrhage by passing needles beneath the vessels which did away with potentially infected sutures and thus eliminated one of the causes of sepsis.[64]

Lister's great contribution has been documented by many surgeons. Thus, Sir Berkeley Moynihan, the great British surgeon, wrote: "His first step was a realization of the truth that decomposition in wounds depends upon the activity of living organisms; his second, which followed immediately was based upon the belief that such organisms might be destroyed in the wound, or as they were about to enter the wound; his third and last, which came more slowly, was founded upon the hope that organisms within the field of operation might be destroyed before they entered the wound. Fierce controversy raged about every step. The hostility and skepticism of early contemporaries was stupid, unimaginative and petty. . . . Lister . . . the man who has changed the face of surgery."[109a]

Harvey Cushing later wrote: "Lister freed man from the shackles of sepsis—he was horrified at the condition in which he found surgery and deliberately elected to crusade against the most serious obstacle in the way of its advance—he was a sensitive man to whom strife was hateful—faced by opposition and misrepresentation, he must make a fight for the truth—it was the students who gave testimony to Lister whom others doubted. Kindness, meekness and comfort were in his tongue."[32]

Rickman Godlee became assistant to Mr. Lister in his private practice in London. The modern refinements of surgery had not been thoroughly worked out and even Mr. Lister was remiss according to our standards. Godlee wrote: "Mr. Lister did not boil his instruments; they were simply placed in a solution of carbolic acid 1:20. He wore no gloves, and his hands, which it should be added were always rough, were sterilized by a mixture of 1:20 carbolic acid in 1:500 sublimate solution (mercury). This

goes by the name of "strong mixture" at King's College Hospital; and it is much too strong for ordinary hands. Lister used marine sponges which were purified in carbolic acid solution. In his early days his sponges after an operation were placed in a tank of water where the small amount of remaining blood contained in them was decomposed. Before the operation these sponges were taken out and washed in carbolic acid lotion and he used to hold that this purification was sufficient."[83]

Lucas-Championnière visited Lister in 1868 and on his return to France gave the world the first systematic account of antiseptic surgery, first in a journal in 1869, and then in a small book in 1876.[84] The French, who should have been in the forefront in adopting the antiseptic system, put many obstacles in the way of those who attempted it. The Germans, however, took it up at once and made great use of it in the war of 1870.

H. A. Marcy was greatly impressed with Lister's work in Edinburgh. He returned to Boston, Massachusetts, where Professor Bigelow, the leading surgical authority in New England, listened patiently and admitted that there might be something in Lister's methods. But he lacked conviction of the truth of its fundamental principles and soon condemned it. Comparatively little progress was made with Lister's technique in New England until the German operators had accepted and improved upon it. Dr. Gouley of Bellevue Hospital, New York City, was bitter in denouncing "the new nonsense."[100]

Ashurst, in apology for the surgeons of Philadelphia who were so slow in accepting the antiseptic principle, wrote that it was always being changed. First, it was carbolic acid, then carbolized oil, then carbolic putty, then watery solutions of carbolic, then a carbolized cerate plaster, then a lac plaster, then oil of eucalyptus. Lister published no statistics. His lists of individual cases were no better than Syme's or those of the Philadelphia surgeons.[7]

The physicist John Tyndall demonstrated clearly in work initiated in 1862 that the air was full of floating dust particles by passing a concentrated beam of light through it. If this dust was allowed to settle under a bell jar, the air then contained no bacteria or yeasts as he showed by exposing infusions of hay, trip, turnip, oyster, etc. to it. No one could any longer doubt that the dust of the air contained germs.[101] Lister introduced the carbolic spray because of this evidence. Soon patients and surgeons developed mild carbolic acid poisoning, numb hands, smoky urine, and irritated eyes. The German Bruns, denounced the spray,[84] but Lister used it from 1870 until 1887, admitting later that he was ashamed that he had ever recommended it. In 1877 he was called to London. Although

his reception was cold and even discourteous, he won over the rising generation as he did everywhere. With Watson-Cheyne he gradually succeeded in convincing even the stolid British.

Wars have always been regarded as great training grounds for surgeons. This may have been the case before antiseptic surgery but after its discovery, wars merely nullified everything that the surgeon had learned in civil life. There were too many wounded to allow the application of the careful treatment that had developed. Pirogoff, the great Russian surgeon, witnessed all the horrors of pyaema, hospital gangrene, erysipelas, and purulent edema in and around Sebastopol during the Crimean War in 1854-1856. He defined war as a "traumatic epidemic," held that large hospitals were responsible for it, and that small, barrack-like pavilions should be built to house the wounded.[31]

Even as late as the 1880's the leading surgeons at the Massachusetts General Hospital, Boston, operated without any evidence that they understood the antiseptic system. Dr. John M. T. Finney, described the operative ritual as he remembered it. It is a wonder that any operation was ever completed without infection. The wooden-handled instruments were placed in metal pans containing 1:20 carbolic solution. Ligature and suture material was treated in the same way. The chief surgeon wore a black Prince Albert coat contaminated with dried blood and wound secretions. He scrubbed his hands in a careless fashion, although his assistants were more careful. The operative field was prepared in a perfunctory manner with soap and water and a dash of bichloride of mercury 1:1000. Towels wrung out of the same solution were used as drapes. The sponges were seasponges kept in seven large glass jars marked with the days of the week and containing 1:40 carbolic acid. The assistant with his bare hand removed the sponges from the jar marked with the particular day and placed them in unsterile agate ware basins. He poured 1:40 carbolic or 1:1000 bichloride over them. At the end of operation the sponges were rinsed in ordinary tap water and returned to the same jar till the next week.[48]

The status of surgery before Lister was in such a bad way that it is a wonder that anyone elected it as a profession. Many surgeons, to be sure, were discouraged and depressed about their results. Pirogoff expressed this in a monograph:[123] "But we shall soon see how often chance and much that is still dark and obscure for us in surgical practice comes so prominently forward that all those qualities—skill, judgement, etc. are completely paralyzed thereby."

Koch's method of plate culture (1882) opened the field for bacterial progress. Koch himself became interested in traumatic infectious disease.

He proposed bichloride of mercury as an antiseptic.[89, 107] Surgeons who had followed Lister's idea on antiseptic surgery decided that the germs must be everywhere if the air was full of them. So quickly there developed methods to kill these germs on everything that might be used in an operation. Gustav Neuber at Kiel boiled the instruments, solutions, dressings, bandages, and even the patient's clothing, before the operation. He introduced caps and gowns in 1882; in 1883, he gave up bichloride of mercury irrigations and used only isotonic sodium chloride solutions; and in 1884, he tried to obliterate the dead space and to do away with drains. His results were so good that other surgeons copied his technique. He wrote ten monographs on antiseptic wound treatment.[111]

Steam sterilizers of various types were developed in Pasteur's and in Koch's laboratories from 1876 to 1881.[106] Ernst von Bergmann (1836-1907) was one of the first German surgeons to try to kill bacteria by systematically treating everything to be used in an operation. Carl Schimmelbusch helped him to develop the steam sterilization and packaging of dressings, solutions etc. in much the same form as they are used today. They introduced bichloride of mercury to replace Lister's carbolic acid (1880). They stressed the importance of hemostasis, steam sterilization for dressings (1886), sterilized nail brushes (1891), added 2 per cent sodium carbonate to increase the germicidal properties and to inhibit corrosiveness of the instruments (1891), and finally published a book in 1892.[12, 139]

Davidsohn (1888) showed that boiling water was a good disinfectant for instruments[33]; Adolph Bergmann and Albert Landerer (1889) emphasized accurate hemostasis, a dry operative field, elimination of irrigation of the wound and a dry dressing[13, 84]; William Halsted introduced rubber gloves (1889-93)[70]; Carl Flugge and R. v. Mikulicz suggested and used masks (1896)[56, 106]; and William Hunter is credited with the introduction of gauze masks at Charing Cross Hospital, London, in 1900.[65]

The trend from antiseptic surgery to so-called aseptic surgery was thus a gradual development. Aseptic surgery was really and still is only refined antiseptic surgery and succeeds only because of the proper respect for the natural repair processes of the human body.

Welch who was studying in Germany at this period brought the knowledge back to the United States. His laboratory at Johns Hopkins became a hive of activity in the study of antisepsis, wound infections, and the improvement of the aseptic system. William Halsted was under his care at this time, and much of the Hopkins surgical technique was developed in Welch's pathology laboratory.[108]

William Stewart Halsted (1852-1922) showed that irrigation of fresh wounds with a solution of corrosive sublimate as weak as 1 to 10,000 was followed by a distinct line of superficial necrosis demonstrable under the microscope. His studies of wounds led to gentle handling of tissues, pin point hemostasis, exact approximation of surfaces, avoidance of dead spaces, and the use of fine suture material. He left his wounds clean and dry. His own account of the introduction of rubber gloves is a human interest story.[70, 97]

> In the winter of 1889 and 1890—I cannot recall the month—the nurse in charge of my operating room complained that the solutions of mercuric chloride produced a dermatitis of her arms and hands. As she was an unusually efficient woman, I gave the matter my consideration, and one day in New York requested the Goodyear Rubber Company to make as an experiment two pair of thin rubber gloves with gauntlets.

The fact that Dr. Halsted married "this unusually efficient woman" shortly afterwards may have had something to do with his giving "the matter consideration." The rubber gloves in use at the Johns Hopkins Hospital and later at the Peter Bent Brigham Hospital, Boston, were rather heavy with ribs down the backs of the fingers. They were used also in the post-mortem rooms at Hopkins. I have been told that the head nurse was the only person to use these rubber gloves when they were first obtained. The idea, then, was to protect her skin against the bichloride solution. None of the surgeons seemed to sense that here was another link in the chain of protection against infection. Dr. Cushing told me that "Joe" Bloodgood began to use these gloves first among the surgical staff and said that he liked them. The others thought him a little queer about this. Once the surgeons appreciated the real value of the gloves they all fell in line. Years later Cushing gave up the heavy gloves and the wet technique with great reluctance.

It soon became evident with bacterial culture methods that even the most careful preparation did not provide a bacteria-free wound. Halsted showed this in 1880.[97] Sir Almroth Wright (1916) studied the antiseptics in general use at that time. He reported that none of the ordinary antiseptics could extirpate the microbes in wounds. He recommended that physiological salt solution 0.85 per cent be used in wounds as it had a positive chemotactic effect on the white blood cells, bringing them to the surface. Wright showed that the white blood corpuscles could not function unless they had a scaffolding such as fibrin to reach the bacteria.[174] His assistants were Colebrook and Fleming and his laboratory was attached to Base Hospital No. 13 in Boulogne, France, where U. S. Base Hospital No. 5 (Cushing's Unit) was assigned.

Halsted stated that America learned its surgery from Germany and that England, in spite of Lister's original work, learned its surgery from America. A uniform technique was adopted in most of the large hospitals in the United States. This technique made it possible for the surgeon to operate on practically any portion of the body. By the time of World War I (1914) nearly all types of surgery had been developed. The surgery of the heart and that of the lung, diaphragm, esophagus, and pancreas were just about ready to start. Infection occurred in a small percentage of cases but it was no longer feared in the approach to any surgical field.

Then came World War I and with it all antiseptic principles seemed to disappear. Tetanus, pyogenic infections, traumatic gangrene, erysipelas, septicaemia, and anaerobic gas bacillus infections became almost epidemic and overwhelmed the surgical attendants. Frantic searches were made for new and more powerful antiseptics. Iodine was found to be too toxic except for surface applications.[62] The Carrel-Dakin treatment, sodium hypochloride, required too much attention to be practical under war conditions.[32, 66] Iodoform as bismuth iodoform paraffin paste had little germ-killing potential although a fine medicinal odor.[109] The mercurials all damaged tissues and were slow in action. Even the beautifully colored mercurochrome[177] with its popular appeal was incapable of sterilizing the skin, infected tissues, or blood.[46, 124]

An outstanding discovery in the study of inflammation was that of Cohnheim (1873)[16, 27, 28] who demonstrated that white blood cells passed into the tissue spaces through the intact capillary walls. Metchnikoff (1884)[17, 104] added to our knowledge when he found that white blood cells and fixed tissue cells engulfed bacteria and destroyed them. The mechanism of arrest and fixation of infecting organisms in an inflamed area was discovered by several investigators.[102, 157, 159]

The aseptic system so carefully worked out by the Germans did not appear completely perfect because a small number of infections continued to occur in clean cases. Carl Walter[166] assessed the causes for failure in the aseptic treatment. He searched for the flaws in the system and pointed out in minute detail where breaks in the technique could occur. This outstanding contribution made it possible to diminish the risks of infection where the slightest slip may lead to disaster.

When an incised wound is made upon the human body there is local damage. This consists in the cells killed by the incision, the blood vessels severed, and the foreign bodies introduced into the crevices of the wound. These foreign materials include the bacteria resident on the skin of the individual, those falling into the wound from the air and those present in the indriven dirt, cloth, metallic bodies, glass or wood fragments. A local

response is immediately set up. The damaged or injured cells take on an acid reaction.[59, 74, 104, 112, 184] The severed vessels react mechanically by retracting and rolling their edges inward.[64] A chemical reaction starts clotting in the vessels with thrombi occluding to the first branches.[94] Collateral circulation develops.[82, 110, 116, 117] The nervous mechanism triggers a sympathetic constriction—all the preceding being defensive reactions toward prevention of blood loss. The introduced bacteria like any invaders seek openings for attack after a short respite. Within an hour bacterial invasion of the blood stream may have occurred, and only after a little longer lapse the lymphatics may also show the invaders.[41, 54, 86, 138, 172, 176] Within two to three hours inflammation will have occurred as an additional body defense. This will consist in dilatation of the blood vessels—increased heat and redness and extravasation of plasma into the tissue spaces.[58, 75] This causes edema and pain, both of which are defensive; edema blocks the vessels by increased tension and pain causes the individual to keep from moving the injured part. Migration of leukocytes through the blood vessel walls into the tissue spaces occurs in the inflammatory stage.[27] Small thrombi form sealing the smaller vessels from invading bacteria. A fine fibrin mesh is organized in the tissues around the injured area.[102] The migrated leukocytes begin to phagocytize the bacteria on the surface of the wound, between the intercellular surfaces, in the clotted plasma and along the fibrin strands.[104, 172] This is one of the decisive acts in the struggle against the bacteria. Amoeboid cells in the connective tissue also take part in this phagocytosis.[104] Some of the white blood cells will lose their lives in this struggle, thereby making the pus which is so evident in a long neglected wound.[131]

While all this is going on locally there is also a general body response—the stress response,[76] which will mobilize the central forces in the struggle. These consist in an increase in platelets and plasma fibrinogen, shortening of the clotting time and a decrease in prothrombin activity.[141] The clotting time rises subsequently. The initial shock will cause pallor and general constriction of the peripheral vessels. This will also minimize the blood loss. If the shock becomes profound, the increase in infection may be 50 times greater,[107] the defense mechanism being removed or destroyed. The adrenal cortical mechanism responds in every case as indicated by the increase in cortisone, the fall in the eosinophil count, and changes in the sodium-potassium ratio in the urine.[76]

The first line of defense against the bacteria is the local one. The second line of defense is the regional one. This consists in the lymph nodes which filter out the bacteria. The nodes become enlarged and tense which pre-

vents extension of the invaders while the blood cells fight them within the nodes.[172]

The third line of defense is the central one. Invaders which have reached the blood stream either by the blood vessels or lymphatics are agglutinated by a nonspecific mechanism essentially in the liver and spleen where they are phagocytized by the reticulo-endothelial cells.[5, 14, 20, 25, 80, 151, 155, 160] The lung may play a relatively small part in this fight as well.[151] The struggle between the body forces and the bacteria is won or lost by the phagocytes. It is a nonantibody mechanism.[172]

When a surgeon is confronted with the problems of treatment for an incised or lacerated wound, the time which has elapsed since the injury is of the essence. There is a safe period during which the growth of the wound bacteria is at a minimum. This makes it possible to treat an accidental wound on the same basis as a planned operative one. There is a fairly general agreement that up to eight hours it is safe to suture civilian accidental wounds after proper treatment.[57, 64, 159] If the patient is seen within this safe period, shock and hemorrhage should be controlled. Then under antiseptic conditions the wound should be thoroughly cleared of all damaged tissue, blood clots, and foreign material. Excision of the damaged edges of the wound should be followed by primary suture.[37, 57, 66, 154] Under the stress of war conditions, the safer course was to leave most of the wounds open. This became the Allied policy in World War I. But it was demonstrated first by Ollier (1872)[157] later by Orr (1920)[114] and conclusively proved by Trueta and Barnes (1940)[159] that complete immobilization after excision was a safeguard not to be neglected. When a plaster cast was accurately applied directly to the skin without cotton or stockinette, the limb was put at complete rest. Even though the wound was contaminated no invasion occurred regionally or generally. The local tissue defense was adequate to control these infections. If there was active muscle movement, or the limb was passively moved, or massaged, or warmth applied to it, the lymph flow would be increased. The bacteria would then spread or their toxic products would be absorbed.[5] This was confirmed by the increase in temperature readings when a plaster cast was changed. Pain, also, became prominent then. Rest for wound treatment was advocated by the Hippocratic school—the supine position being recommended.[95] McMaster (1937),[99] showed that there was little lymphatic flow of vital dyes in the horizontal position.

When a wound is over 12 hours old, it can no longer be considered a fresh wound. The problem at this juncture is different. The bacteria have had time to multiply in the wound, and the local tissue defense has resulted in an inflammation.[9, 70, 87] The inflammation poses a barrier to the bacteria.

Although it does not constitute a rigid barrier to passage of lymph, absolute rest of the area will reduce the hazard of the spread.[159] This fixation of bacteria and irritants has been noted by several investigators. It takes place very early in the inflammatory process. Foreign substances, bacteria, dyes, metallic salts, particulate material injected into an inflammatory zone remain *in situ* and fail to drain into tributary lymphatics.[102] The same materials injected intravenously accumulate rapidly in the inflammatory area because of the increased permeability of the capillaries. But these substances cannot escape from the inflammatory zone because of a fine network of fibrin and thrombosed lymphatics.[102] The accepted treatment for these older wounds was to leave them open to drain and to granulate after excising dead or devitalized tissue without surgical insult.[87] They were splinted whenever this was feasible with apparatus such as the Thomas and Aeroplane splints or plaster with windows so that access to the wound dressings was easily available. A considerable period of treatment was required following this practice and only when the granulations were beefy and cultures showed no streptococci was it safe to do secondary wound incision and closure. Secondary closure when streptococci were present in large numbers was invariably followed by breakdown of the suture line after a febrile reaction. Here again complete immobilization of an inflamed wound will reduce the chances of bacteria passing out into the general circulation. The casts could be left in place for weeks if necessary as long as the smell was not excessive and the casts were not soft and wet. Leriche suggested the use of Brewer's yeast in the dressing to modify the odor.[159]

In old wounds a layer of granulation tissue is formed. Welch wrote in 1895: "The surface of a healthy granulating wound offers great resistance to the invasion of bacteria almost as much as an intact exposed surface of the body. Slight injuries, however, such as probing, the removal of dressings, and other manipulations may convert a granulating surface into a fresh wound with the accompanying dangers of infection."[160]

Halsted introduced gutta percha protective in 1880, probably because of Welch's influence. In World War I vaseline gauze was substituted, a single layer over the granulating surfaces not disturbed unless it came away with the dressing during change. Halley, Chesney, and Dresel tested the generally accepted view that granulation tissue was resistant to bacterial infection. They found that granulation tissue in rabbits constituted a relatively unfavorable environment for the survival and growth of streptococci and staphylococci. However, Pasturella avicida (fowl cholera) readily passed into the circulation and killed the animals.[98]

Nobody, to my knowledge, has commented on the relation of granulations to time. At a certain period in wound healing fixation occurs and generalized infection is no longer possible. When the granulations became exuberant, growing well above the epithelial edges it was common practice to burn them down with a silver nitrate stick or to cut them off with scissors. Granulation tissue has no nerve supply and is not sensitive. No septicemia resulted after such treatment. Dr. Cushing used to trim down the granulations in old wounds with scissors. Many times the plastic surgeons scrubbed away the granulations with a stiff scrubbing brush before placing a skin graft. No general sepsis followed.

The dead space has been of interest to surgeons as one of the difficult problems in healing. It is a hollow cave in the tissues which has no blood supply. It acts as a reservoir for serum and as the white blood corpuscles cannot reach the bacteria in it, it becomes infected. Della Croce[38] in 1560 warned against leaving dead spaces. Neuber (1882) tried to obilterate the dead space and to do away with drains.[111] Halsted was especially concerned with the dead space as it became a challenge following his radical breast operation. The removal of the pectoral muscles left a decided hollow below the rigid clavicle and this area filled with serum if the skin flaps were approximated by pulling them together. This may have been one of the reasons why he developed skin grafting of the area. He told me on my return from France in 1919 that he had at length conquered the dead space. He said "I pull the wound apart and not together, that is the answer to this problem." The recent use of negative pressure and suction drainage under the skin flaps after radical mastectomy is an advance in the management of the dead space.[128]

Abscess cavities are dead spaces. In the center of established abscesses phagocytosis is ineffecitve. This is due to insufficient contacts between phagocytes and bacteria. Lack of oxygen makes the phagocytes nonmobile and thus they lose their phagocytic properties. Natural resistance to acute bacterial infection is significantly lower in cavities—even the most rigorous chemotherapy will rarely cure an established abscess unless some form of drainage is used.[127] Every surgeon knows this only too well. If the abscess has not become chronic, aspiration of the contents or drainage will suffice to allow the walls to collapse and approximate. The white blood cells can then phagocytise the bacteria and overcome the infection. If the abscess has a thick wall of fibrous tissue, it may be necessary to do a plastic repair, or to excise the whole area. The dead space left in bone after evacuating an osteomyelitis focus was an intriguing problem. Such a burned out area was sterilized by antiseptics and allowed to fill with a blood clot by Schede.[127] Halsted was likewise concerned with this problem. He told me

that he used it as a test of the technical skill of the residents that he had trained. Not all of them could get healing on the blood clot.[89]

Another cause for dead spaces in wounds is high velocity projectiles. When such missiles strike the body they impart their kinetic energy to the tissues and fling them away from their path in a radial direction. This results in a temporary cavity around the wound tract. The wound of exit shows a splash-back effect—it is often larger than the wound of entrance. The missile frequently turns sideways on coming into contact with a medium of different density.[148]

The German dye industry recognized that the sulfonamide group (NH_2SO_2) made an excellent mordant when it was introduced into their acid wool dye stuffs. Its presence in the dye insured a strong affinity for the protein molecules and guaranteed the fastness of the dye. Among the many synthetic dyes made was one p-aminobenzene sulfonamide put together in 1908. It was recorded in an obscure journal and its significance was not recognized for 27 years.[21] Mietzsch and Klarer in 1935 made some dyes for the I. G. Farbenindustrie and they were patented.[21] In the search for antiseptics, many dyes had been found to be bacterial.[26, 84, 142] Domagk tested one of these dyes—prontosil against mice intraperitoneally inoculated with streptococci and found that they were protected.[40]

Trefouel *et al.* fed the material to animals and found that it was excreted in a colorless form in the urine. This metabolic product was identified as a sulfonamide. Knowing that the aromatic amines were excreted as acetyl conjugates, they realized that the active constituent of prontosil was sulfanilamide xxxiv-5. They prepared this compound and treated infected animals with it. Their experiments indicated a high *in vivo* activity for animals.[150] Colebrook and Kenny applied this knowledge with success to patients and published their results.[29] Here was a discovery of the highest significance. As with any new discovery this one required careful appraisal over the next few years. Here was a drug which could neutralize many of the infections which had passed the barriers into the general circulation. There were occasional complications from the use of prontosil but these were soon catalogued. I can well remember the cyanosis sometimes associated with prontosil therapy.[93] This was accepted in those days as being much the lesser of evils—the general infections in many instances being of a fatal type. Sulfanilamide remained for several years the most important chemotherapeutic agent against the bacteremias. It inhibited the growth of many bacteria of both gram positive and gram negative types.

Many new forms of sulfa drugs were synthesized and put into clinical use. Toxicity was gradually reduced until there were fewer toxic side reactions. Sulfapyridine, sulfathiazole, sulfadiazine, and sulfasuxidine fol-

lowed each other with varying qualities to recommend them in defined circumstances.[127, 171]

The mechanism by which these drugs acted was studied. It was found that some of them in low concentrations actually stimulated bacterial cell division but that at higher concentrations they inhibited bacterial growth. They did not kill the bacteria *in vitro* but were bacteriostatic. Their action followed a lag period. Lockwood indicated that proteolytic products such as peptones counteracted the effects of sulfanilamide.[93] Woods 1940[178] pointed out that p-amino benzoic (PAB) acid was the most powerful sulfonamide antagonist. PAB is needed for the synthesis of folic acid. Folic acid presumably presides over the synthesis of methionine, xanthine, and serine. Vitamin B-12 takes part in these same reactions and all involve single carbon transfers. Folic acid and B-12 are, therefore, probably the co-enzymes or catalysts for the transfer of formyl groups from one compound to another. The mammalian host takes in preformed folic acid. The bacterial parasites do not and have to synthesize it. The sulfonamides act at this point—they interfere with the utilization of para-amino benzoic acid (PAB). In this way they block the fundamental pathway to the formation of the amino acids necessary for the growth of the bacteria.

Arguments pro and con soon divided the medical profession on how to use the sulfa drugs. Some believed that the sulfa drugs could quickly act if put locally in infected areas. Others claimed that the action depended on the general concentration throughout the whole body. The latter viewpoint prevailed after several years.

Fleming continued his studies on cultures and antiseptics after his World War I experience in Wright's laboratories. In 1928 he noticed that some colonies of staphylococcus became transparent when a contaminating mold overgrew them. He identified the mold as *Penicillium notatum*. He found that it had a similar lytic effect on streptococci. He made some crude extracts of the mold and called attention to its possible value in the treatment of pyogenic infections. He named the extract from the mold Penicillin. His publication in 1929 did not arouse any interest in the medical world.[51] In 1941 he explained in a letter to the *British Medical Journal* that "the trouble making it seemed not worthwhile . . . A few tentative observations had been made on the effect of the local application of the unconcentrated culture to carbuncles and sinuses. Although the results were considered favorable, there was no miraculous success. In those times septic wounds in hospital wards were relatively uncommon, and it was not considered that the production of penicillin for the treatment of these was practicable, owing to the lability of the active principle in solution."[21, 50]

In 1941 Dubos and Hotchkiss were studying soil bacteria at the Rockefeller Institute. They found that a Bacillus brevis contained two agents—gramacidin and tyrocidine. Both of these were crystalline polypeptides but were too toxic for use against infections except in local applications.[49, 101]

These growth inhibitors or bactericidal agents which were formed by *living* organisms were called *antibiotics.* Under the stimulus of World War II, the antibiotics were soon to become one of the great international developments. In 1941 Florey and his co-workers at Oxford found that not only was Fleming's penicillin useful as an antiseptic when applied locally, but was also in fact a *systemic chemotherapeutic agent.* It had so little toxicity to animals relative to its antibacterial power that it could be present throughout the blood stream and tissues of the animal in sufficient amount to ensure the elimination of invading organisms. The tissues of the animal were not damaged nor the animal killed by the toxicity of the agent. Chain and Florey decided to apply to the Rockefeller Foundation for funds to promote an academic study of this material. It is of interest to note how meagre their data were in these days of statistical evaluation. Their first tests were upon 8 mice given hemolytic streptococci intraperitoneally—4 mice served as controls, all died within 16½ hrs. The other 4 mice were protected by the penicillin. One lived 2 days, 1 lived 6 days, 1 lived 13 days, and the fourth lived indefinitely. On this basis the Foundation gave them a grant which enabled them to sign up 23 workers as the Oxford group. After further testing on 26 mice, the group decided that this experiment needed to be done on a large scale by larger drug houses. So Florey and Heatley came to the United States in 1941. They spent their first few days at New Haven, Connecticut, with Dr. John F. Fulton and posed the problem to him. All English pharmaceutical houses were under fire and under pressure, but America had not yet entered the war and was safe from attack. Dr. Fulton referred the Oxford men to Dr. Ross Harrison, President of the National Academy of Science. Through the influence of these two men at Yale, Merck and Co., Squibb, and Pfizer agreed to take over the problem. The other pharmaceutical houses declined with thanks. The rest is history; the active material was made in quantity, it was purified and standardized. Six types were evolved. They were tested on bacteria, on animals, and finally clinically. An international unit was established.

Penicillin turned out to be remarkably nontoxic to man, the LD_{50} dose being 1 to 2 gm/kg. body weight. It was especially effective against both gram positive and gram negative cocci, including pneumococci, streptococci, meningococci, gonococci. It was also effective against the Clostridium of gas gangrene, and B. anthracis and against the Treponema of syphilis and

yaws. In rapid succession other antibiotics with wider bacterial spectra were developed. Streptomycin, chloramphenicol, the tetraclines, and neomycin were found to be effective antibiotics against most bacteria, Rickettsia, Treponema, and some viruses.[55]

Agents as valuable as the antibiotics were eagerly applied by surgeons to many of their problems of infection. These antibiotics seemed to be the answer to prayer in the struggle against sepsis. With their use the dreaded invasion of the blood stream could be neutralized.

At first it appeared that surgery was to be freed of all its careful antiseptic precautions. Some trained surgeons regretted that they had put so much time into learning the surgical principles. "Now anyone can pick up a scalpel, operate and give the patient a handful of antibiotics. No training is required and the results are as good as anyone can get." It was not long, however, before it was evident that the underlying principles of good surgery could not be so easily scrapped. The antibiotics had a place but so did the surgical principles. "Antibiotics simply attack the bacteria, the surgeon must treat the patient."[127] The antibiotics are aids to surgical competency. As with the sulfa drugs the antibiotics are therapeutic adjuncts to good surgery but in no wise replace it. Thorough treatment of wounds by skilled surgeons explained the lowered mortality in battle injuries of World War II.[185] The skill of the treatment determined the outcome.[127]

The selection of the proper antibiotic and the many differing conditions which lead to success or failure have been documented.[55, 127] Physicians must know the virtues and the drawbacks of such antibiotics as they may prescribe. Medical attendants must be especially careful not to reinfect their patients.

There was a period in surgery when the millennium appeared to have been reached. The hospitals in the late 40's and early 50's were free of acute infections such as erysipelas, deep cellulitis, carbuncles, Ludwig's angina, lymphangitis, parotitis, osteomyelitis and septicemia. When osteomyelitis started to appear, it could be aborted by antibiotic treatment. Acute empyaema no longer occurred, and chronic empyaema became a thing of the past. Sinus infections and mastoiditis disappeared.

In the early 1950's, however, it became evident that pyogenic infections were becoming more frequent in hospitals. The antibiotics in current use did not seem to be effective any longer. The staphylococcus was the organism chiefly involved and it had become antibiotic resistant. It was found that a high percentage of the attending staff were carriers of these micrococci in the nasopharynx or upon the skin.[24, 77, 182, 148] The nasal carriers varied with the environment and were either persistently positive or persistently negative.[68] The possibility of cross infection in the wards was

recognized.[30, 133] Many newborn babies in hospitals became nasal carriers in 7 days.[148] The avenues of infection were sought and found to be many. The increased infection rate in accidental wounds and in supposedly clean wounds became a cause for alarm.[22, 77, 78, 79] It was found that with every dressing on an open ward thousands of subvisible particles were released into the surrounding air. Making a bed caused a similar disturbance.[30] Cultures from the dust, bedside tables, sheets, mattresses, basins, lavatory seats, and especially blankets were positive for infecting organisms.[30, 129, 149] Woolen blankets were never sterile in some hospitals.[30] Mattresses gave positive cultures many months after storage.[129] An interesting experiment with a fluorescent powder placed on a patient's leg in a bulky dressing showed that it passed through the dressing to the sheets and mattress. And it also went through the surgeon's trousers, gown, and drapes during operation.[129] The infection from the nasal carrier did not appear to be a droplet infection but rather an infection from the nasal secretions soiling the hands, handkerchiefs, clothing, and bedding.[72] Air currents caused by shaking clothing, spattering of water, passing people, machinery, sweeping and mopping were responsible for spread of infection.[72, 149] In the operating room there should be no traffic stream. Sterile plastic aprons have been suggested for the team, impervious masks, and in addition no talking during the operation.[22, 129] Silence here is golden and cleanliness is surgical godliness.[22] No woolen blankets should ever go to the operating room.[22, 30] Oiling the floors, misting the air with triethyleneglycol or other chemicals have been recommended to cut down the dust menace.[30] All dressings should be made before sweeping and cleaning of the wards is underway.[22]

When antibiotics have been used freely and often in a community, the common pyogenic organisms develop resistant strains. The naturally occurring resistant organisms survive.[148] It has been found that the mechanism of survival is different from that of the resistant strains of Trepanosomes. Ehrlich[175] found in that case that the sensitive strains absorbed arsenicals into the cell body but the resistant strains had loss of permeability. There was no difference in the absorption of radioactive penicillin by sensitive and resistant staphylococci. A few penicillin resistant strains were resistant because they possessed penicillinase.[132, 175] Many streptomycin resistant bacteria were also streptomycin dependent.[55] Differences in the strains of staphylococci became manifest. The majority of the strains isolated from human infections elaborated an extra-cellular enzyme—coagulase. This enabled the coagulase positive strains to clot the plasma and thus protect the bacteria from phygocytosis. They grew well in 24 hours in undiluted human serum, whereas the coagulase negative strains were completely inhibited.[45] The coagulase-positive staphylococcus became a menacing organ-

ism. Under certain circumstances it could produce a necrotin for the skin, an enterotoxin which caused severe enteritis, a leukocidin which neutralized the effects of the white blood cells, a hyaluronidase which made it possible to spread through the collagen barrier, and a fibrinolysis which dissolved the protective fibrin clots.[145] When once the coagulase positive staphylococcus reached the blood stream the chances of recovery were only 50-50.[146] These resistant strains have been especially lethal for infants and the aged. The coagulase-positive staphylococcus has replaced the pneumococcus as a bacterial cause of death. No antibody response has been demonstrated although some degree of immunity seems to be acquired.[132] Diabetics have an especial susceptibility to staphylococcus infection because of inadequate nutrition and dietary deficiencies. The diabetic white blood cell produces less lactic acid from glucose than the normal white blood cell.[132] Lactic acid is significantly diminished in the presence of ketone bodies.[42]

The development of new antibiotics such as Vancomycin, Ristocetin, Kanamycin, together with chloramphenicol and erythromycin, offer hope against these resistant strains.[9, 24] The combination of antibiotics with gamma globulin have been tested in both animals[49] and humans[105] with good results, less antibiotic being required under such conditions.

The struggle against sepsis is still with us. It has been a long hard struggle with successes and disappointments. It has been a struggle between bacteria and human resistance. It has been a struggle between men and ideas. We may yet resolve it.

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EVENTS

HARRY BURR FERRIS MEMORIAL LECTURE

April 21, 1959

THE PATTERNS, FUNCTION AND SIGNIFICANCE OF THE ADDITIONAL MOTOR AREAS. By Elizabeth C. Crosby, Professor of Anatomy, The University of Michigan Medical School, Ann Arbor, Michigan.

Fine movements of parts of the contralateral extremities (such as fingers and toes) and of the face on the opposite side or bilaterally are obtainable on stimulation of the so-called "motor cortex," and voluntary eye deviations are functions of portions of the middle frontal and adjacent cortical areas. However, patterns of body movement have been elicited by stimulating areas other than those cortical areas just mentioned, e.g., the amygdala, the cingulate gyrus, the anterior pole of the temporal lobe, and virtually all of the cortex except certain primary sensory projection areas (such as those concerned with vision and hearing). Even the somatic sensory projection area (fields 1, 2, 3) have been demonstrated to have motor functions. Motor responses from the additional motor areas have been obtained under light anesthesia using the same frequency as for voluntary motor responses, although the voltage required is usually slightly higher for the former regions. These additional areas have been demonstrated in primates and, in many instances, in man. Eye movements are seen after stimulation of various regions. Quite probably the additional motor areas are focal or trigger points which figure prominently in epileptic seizures, especially of the Jacksonian type.

Discharge is via multisynaptic paths through the basal ganglia, or directly into the tegmentum of the midbrain. Lesions of the pyramidal system often do not involve extrapyramidal pathways from additional motor areas because of different courses. For example, one set of additional pathways courses through the external capsule, while the pyramidal tract goes through the internal capsule. The variations among the outflow routes of the additional motor systems account for the varied responses to electrical stimulation observed, because patterns of movement are determined by arcs and interconnections rather than by neuronal arrangement in the cortex. This provides a neuroanatomical basis for the variety of movements man can execute, which contrast sharply with the stereotyped movements seen by early investigators stimulating the "classical" motor cortex. Both supplementation and substitution for functions of the motor cortical paths are provided by the additional areas, the importance of which become obvious when diseases like cerebral palsy destroy great parts of the primary motor system. Rehabilitation of such patients often depends upon the integrity of extrapyramidal function. Fine movement may be irrevocably lost, but the gross movements which remain are often adequate to achieve very considerable motor function.

Voluntary movements are colored and enriched by associated movements, and these latter are mediated through the additional motor pathways. One type of cortical (but not necessarily conscious) movement is "following"

eye movement. In such a deviation, the eyes follow a steady movement between two points. These deviations may become conscious, however, if the person's attention is brought to them. "Voluntary" eye movements are those eye deviations from one discrete point to another with no line or moving object connecting the two points, and are cortical and conscious. Emotional and intellectual processes are transformed into overt behavior through associated movements. Such overt behavior is the basis upon which the judgment of the personality of an individual is made by his fellows. Parkinsonian "poker" facies emphasizes through contrast the extreme importance in normal behavior of expressive, involuntary motor activity accompanying any voluntary muscular movements.

M. S. M.

BIOPHYSICS SEMINAR

April 27, 1959

BIOLOGICAL MEMBRANES AS REVEALED BY TRANSPORT STUDIES. By Arthur Solomon, Harvard University, Cambridge, Massachusetts.

Recent experiments on the human red cell membrane have provided new evidence on how ions and molecules enter and leave cells. For a number of years the presence of either pores or carrier molecules has been postulated as the means of transport across the protein-lipid cell boundary.

Ion flux studies indicate that the Cl^- $t\frac{1}{2} = 0.1$ seconds, while the K^+ $t\frac{1}{2} = 45$ hours. This million-fold differential is best explained by assuming, (1) that a positive charge barrier in the pores inhibits K^+ flow, and (2) that the pore size is somewhere near that of a K^+ ion (10-20 times as large), so that the abundance of Cl^- ions present might sterically hinder K^+ transport.

Using tritium-labelled water, diffusion gradient and osmotic pressure gradient flows have been measured (0.62×10^{-14} and 1.5×10^{-14} mH_2O/sec., cm H_2O, red cells, respectively); since the two values are not equal, it is thought that water-filled channels must pass through the cell membrane. From this data an ideal "equivalent pore radius" may be estimated as about 3.5 Å. This surprisingly low figure is indirectly supported by the fact that only organic molecules with three carbon atoms (radius ca. 2.8 Å), or less, may enter red cells.

Further studies with varying tonicities show that when an erythrocyte swells by 10 per cent, the rate of water diffusion across the membrane falls by 27 per cent. This suggests that the pores may be occluded as the membrane swells, perhaps because a small charge could produce a relatively large effect in such restricted areas. It is particularly noteworthy that with the 10 per cent swelling, the K^+ transport only dropped by 3-4 per cent, a finding suggesting that K^+ enters the cell by the carrier molecule mechanism.

N. K. W.

May 5, 1959

SOCIALIZED MEDICINE. By Mr. James Brindle, Director, Social Security Department, United Automobile Workers, Detroit, Michigan.

Mr. Brindle was introduced as the Social Security Director of the UAW, and the former acting director of the Community Health Plan of Detroit, which Dr. Frederick D. Mott now heads. His remarks on "socialized medicine" were confined mainly to the effect that the term "socialized" has lost most of its scare value. He then went on to discuss the community-sponsored prepayment health plan which the UAW favors.

The pressure behind the inception of this plan was stated to be due to increasing recognition of the inadequacies of the medical insurance schemes at present available. In general, such insurance has provided for only part of some medical expenses, and not at all for many others. In view of the constantly increasing cost of medical services it has become a major economic problem for the union, as well as being a general social problem.

One of the factors which has contributed to the present unsatisfactory state of affairs was attributed to the faculty base of most medical insurance plans. The insurance companies have applied their usual business principles to the finances of medical insurance, and have paid no attention to the quality of medical care available. Their aim has been to limit the number of claims which may be made, rather in the manner of automobile insurance. The end result has been an increase in hospitalization rates, as most plans have required hospitalization as a prerequisite for payment of benefits. In the opinion of some union leaders this is a major factor contributing to the increasing cost of hospital care. Mr. Brindle cited hospitalization rates (bed days per 1000 population covered per year) of members of the Kaiser Plan in California, contrasting them with Blue Cross rates. Participants in the Kaiser Plan, who are not required to be hospitalized in order to obtain benefits, have a rate approximately one half that of subscribers under many Blue Cross plans.

In Detroit, UAW is backing a prepayment scheme which will cover complete medical expenses of good quality medical care. Most of the physicians will be full time, in hospital-centered medical groups. It is hoped that a good doctor-patient relationship can be maintained in group practice. The declared intention is to have the plan controlled and policed by physicians. It is expected that this plan will operate on a sound economic basis with premium rates not much higher than the present Blue Cross-Blue Shield premiums. The rate will be flat, retired workers will retain their membership, and membership will not be limited to union members.

Mr. Brindle said that there has been opposition to the scheme, and that it has not been easy to obtain the active participation of many physicians. He was optimistic about the final outcome, but felt that the medical profession could well supply leadership now. In default of this, it was quite apparent that the guiding force would come from labor and other lay community leaders. He suggested that medical schools become more active in the development of prepayment programs.

PRESENTATION OF THESES OF CLASS OF 1959

May 21, 1959

THE FIBRINOLYTIC ACTION OF HEPARIN. By Lyall S. Crary.

In this thesis a review of the literature concerned with the fibrinolytic action of heparin is presented as well as detailed results of *in vitro* and *in vivo* experiments. The *in vitro* work generally indicates that heparin has a definite fibrinolytic action which is more pronounced in plasma than in serum, which is dependent upon some labile factor in plasma or serum, and which is directly related to the concentration of the heparin. The *in vivo* experiments suggest that the intravenous administration of heparin induces an increase in the fibrinolytic activity in the circulating blood. Other work purports to show that heparin has a definite thrombolytic action *in vivo*.

Because of the nonspecific methods used by previous investigators, some of the experiments dealing with the fibrinolytic action of heparin are repeated in this thesis using a more accurate and specific method of measuring fibrinolysis. To summarize the method, standard fibrin clots are made from bovine fibrinogen trace-labelled with Iodine-131 diluted with human or dog plasma. After complete retraction and washing to remove nonclottable radioactivity, the test clots are immersed in the test solution for the desired length of time. If an active fibrinolysin is present, the fibrin dissolves and, as it does so, the radioactivity is released into the supernatant. By counting the radioactivity appearing in the supernatant and that remaining in the undissolved portion of the clot with a scintillation counter the per cent of radioactivity released from the clot, and thus the per cent lysis, can be calculated. A preliminary experiment using streptokinase and plasmin demonstrates the reliability and accuracy of the method.

A series of *in vitro* experiments are presented which fail to corroborate the findings of previous investigators. Heparin in a concentration of five to 500 gamma per milliliter of fresh human plasma does not produce fibrinolysis above control levels. Experiments designed to bring out a hidden fibrinolytic action are also negative.

In vivo experiments are presented in which the plasma from serial blood samples drawn from the heparinized dog and heparinized human is incubated with the test clot for 24 hours. No significant increase in the circulating fibrinolysin in the plasma could be demonstrated.

It is concluded that heparin does not have a significant fibrinolytic action *in vitro* or *in vivo*, and that the beneficial effects of its use in thromboembolic diseases must be attributable to its other properties.

ANTIGENIC ALTERATION FOLLOWING HETEROTRANSPLANTATION OF THE MOUSE S-37 TUMOR. By David B. Skinner.

The feasibility of heterotransplanting tumors has been an experimental fact for the last 20 years, but the differences in biological properties of these tumors from those which do not survive transplantation has been incompletely explored. Two general theories of cancer biology, proposed

by Dr. H. S. N. Greene of Yale and by Dr. H. N. Green of Leeds, are based on the hypothesis that alteration in the antigenicity of the tumor cell is a prominent part of the neoplastic process and is responsible for the survival of the heterotransplanted tumor. Further investigation of this hypothesis was attempted by studying possible antigenic changes in the heterotransplantable mouse tumor, S-37, by the immunological technique of anaphylaxis.

The S-37 tumor was carried in DBA mice and heterografted to guinea pigs and hamsters. Extracts of the mouse S-37 were used to sensitize stock guinea pigs, and these sensitized animals were later challenged with similar extracts of the guinea pig or hamster S-37. If no anaphylactic response occurred, the challenged animals received a second injection of the original sensitizing mouse antigens to determine the actual state of sensitivity of the animals.

By this technique, 29 mouse S-37 sensitive guinea pigs did not react to challenge with guinea pig S-37 extract, and 7 mouse S-37 sensitive guinea pigs did not react to challenge with hamster S-37 extract. Hence it was concluded that antigens detectable in mouse S-37 could generally not be detected following heterotransplantation of this same tumor. Studies of the immunological responses of the heterotransplantation hosts were also compatible with this conclusion. However, this study did not necessarily prove that a change occurred in the tumor cell antigens themselves at the time of transplantation, as it remains uncertain whether tumor cells contain species-specific antigenic substances at all.

Similar experimental studies by Watson, by Maculla, and by Korngold and Lipari were discussed and were found to be in general agreement with the above findings. Since antigenic changes had been observed in a total of five tumors studied by three different immunological techniques in these four studies, it was further concluded that a loss of antigenicity seemed to be a definite biological property of autonomous neoplasms. Thus both theories of carcinogenesis proposed by Drs. Greene and Green received experimental support from these studies. The theories were discussed and seemed to be compatible and supplementary to each other.

BOOK REVIEWS

THE CHEMICAL BASIS OF DEVELOPMENT. W. D. McElroy and B. Glass, Eds., Baltimore, The Johns Hopkins Press, 1958. 934 pp. $15.00.

The 41 papers that compose this volume were presented at the McCollum-Pratt Institute Symposium in the spring of 1958. They fall into four broad categories entitled Developmental Cytology, Cellular and Tissue Interactions in Development, Problems of Specificity in Development, and Control Mechanism in Development. Many of the current leaders in developmental biology contribute to the book either as author or discussant. In some respects this is not as desirable as might be supposed since many of the well-known participants have published the bulk of the material reported in this volume in one or several of the other symposia that have appeared in recent years. Because of this duplication the present volume would hardly be worth possessing were it not for the inclusion of about six outstanding papers and some penetrating and thought-provoking discussions. It is hoped that this will be the last symposium on developmental biology published until more new and significant advances are made in the field; it would be fitting in many ways to pause with this book in hand, for it does treat almost all of the great variety of subjects that interest contemporary researchers—a feat not often accomplished by a single volume.

As the title implies, the predominant theme is the interpretation of developmental phenomena in chemical terms. The success achieved in this regard varies with subject and author; unfortunately, too many biologists have a superficial knowledge of contemporary biochemistry. A typical example, on the other hand, of a truly productive integration of modern chemistry and developmental studies is the A.D. Glinos study on liver growth and regeneration. In contrast to this is the Yamada work on inductor substances extracted from dead adult tissues. From the point of view of chemistry this is a first-rate contribution, but with regard to normal amphibian induction, as Holtfreter points out in the discussion following the paper, the work is at best of peripheral interest. From this and other papers in the volume, one gains the impression that certain developmental biologists have become so enamored with techniques that they have lost sight of the integrated biological system that should be the focal point of any information gained. It is encouraging that several authors (as S. C. Shen) caution the molecular biologist to pause and re-examine his experimental design and results in these terms.

The book contains adequate author and subject indexes. It is well bound but printed on rather thin paper. The illustrations vary greatly in quality, some being quite inferior. The bibliographies of individual papers are excellent, although titles are not given. On the whole the inadequacies do not outweigh the advantage of having a single reference volume for all of modern developmental biology.

N. K. W.

THE MIDDLE EAR. By Heinrich G. Kobrak. Chicago, The University of Chicago Press, 1959. 254 pp. $15.00.

Rosen's revival of staples mobilization in 1952 for the treatment of otosclerosis served as a powerful stimulus to the otolaryngologist's interest

in middle ear surgery. Concurrent improvements in the operating microscope made it possible to visualize middle ear structures in amazingly clear detail, and revealed to the ear surgeon the possibilities of preserving or restoring hearing in the surgical treatment of chronic middle ear infections.

Since 1952 many articles have appeared describing various techniques of reconstructive surgery of the sound-conducting apparatus, and a few others have discussed the physiological principles on which these procedures are based. The present work serves an important need in providing the otologic surgeon with a thoroughgoing analysis of the technical and physiological aspects of middle ear surgery in one comprehensive volume. It describes in detail the experimental methods used in investigating middle ear function in the laboratory. A brief review of the pathology and physiology of the middle ear is included and there is a concise presentation of acoustic physics in understandable language. Finally, a clear, well-written section on treatment covers the newest techniques in the surgery of middle ear deafness. These include stapes mobilization, fenestration, prosthesis and tympanoplasty, each section written by a leading authority in the field.

The reader might regret that nonsurgical methods of treating and preventing middle ear disease and deafness are not included. The average otolaryngologist consulting a volume by this title might expect to find a section on middle ear effusion since it is a frequent cause of conductive deafness and often requires surgical treatment. These topics are apparently outside the scope of this volume. The sections on testing and on pathology are rather sketchy but the otologic surgeon looking for a well-written, clearly illustrated description of the newest techniques in microsurgery of the middle ear will find this book a valuable source of information.

JOHN A. KIRCHNER

REPRODUCTIVE PHYSIOLOGY. COMPARATIVE REPRODUCTIVE PHYSIOLOGY OF DOMESTIC ANIMALS, LABORATORY ANIMALS AND MAN. By A. V. Nalbandov. San Francisco, W. H. Freeman and Company, 1958. xi, 271 pp. $6.75.

This work is very brief and is written in an elementary style for people interested in veterinary problems for the most part. There are a few basic facts which apply to birds, rodents, and man respectively, but the emphasis throughout the book reveals the author's primary interest in birds and cattle. It is regrettable that he did not see fit to use or quote the sterling research of F. L. Hisaw, Edgar Allen, C. G. Hartman, G. Corner, H. M. Evans, and so many others who were responsible for giving great impetus to the establishment of the endocrinology of reproduction as a scientific discipline. Most of the references are secondary source materials (reviews, monographs and textbooks). The brevity of the present work is admixed with numerous categorical and telegraphic statements, *viz.* "The relative potencies of these compounds (estrogens) have been considered to be α estradiol > esterone > β estradiol > estriol." First, it should be pointed out that alpha estradiol is a misnomer if the author is referring to estradiol-17 beta, and that β-estradiol is actually alpha estradiol. The nomenclature for the estrogens was corrected in 1949 by Feiser and Feiser. Secondly,

alpha estradiol has only 1/200 the potency of estradiol-17β in the vaginal cornification bioassay. Furthermore, one cannot make general statements about potencies of estrogens, especially since each estrogen is potent for a specific metabolic end-point. Finally, it should be pointed out that modern endocrinology leans heavily upon biochemistry and mechanisms of actions. In both of these respects this book is lacking. One will find a more thorough account of many aspects of this book in recent freshman college textbooks of biology, zoology, and physiology.

JOSEPH THOMAS VELARDO

HORMONES IN BLOOD. Ciba Foundation Colloquia on Endocrinology, Vol. XI. G. E. W. Wolstenholme and Elaine C. P. Millar, Eds. Boston, Little, Brown and Co., 1957. xi, 416 pp. $9.00.

This new symposium, held under the impeccable auspices of the Ciba Foundation, represents another landmark in the scholarly aims of biochemically oriented endocrinologists. Three main themes are explored *in extenso* in this volume: (1) biological assays of hormones in blood, particularly from the pituitary, thyroid, adrenals, and pancreas; (2) ways in which hormones are transported in blood; and (3) methods employed for the measurement of hormones in blood.

Of particular interest to this reviewer were the chapters on neurohypophysial hormones (by H. Heller), assays of anterior pituitary and placental hormones (J. A. Lorraine), thyroid hormones (Rosalind Pitt-Rivers), factors influencing ACTH (G. Sayers), circulating steroid hormone levels in relation to steroid hormone production (W. H. Pearlman), steroid interaction in the *in vitro* biosynthesis of steroid-protein complexes (Clara M. Szego), progesterone and related steroids (R. V. Short), and iodine in blood (E. B. Astwood, C. E. Cassidy, M. S. Raben, and Sara M. Astwood). Each chapter reaches a high level in its stimulating and scholarly discussions.

This volume will be of great value to the endocrinologists concerned with the physiology and biochemistry of the hormones. Like most scientific investigations, it provides answers and asks numerous questions, and these are good questions.

JOSEPH THOMAS VELARDO

VIRAL AND RICKETTSIAL INFECTIONS OF MAN. 3d ed. Thomas M. River and Frank L. Horsfall, Jr., Eds. Philadelphia, J. B. Lippincott Co., 1959. xviii, 967 pp. $8.50.

Frequent revisions of this standard reference work on medical virology have been necessary to keep pace with the development of the field. Whereas approximately 70 human viruses were covered by the first edition in 1948, over 160 are described in the present revision.

This third edition has continued the expansion, started in 1952, of the section on fundamental virology. The excellent chapter on "Virus-Host Cell Relation" by G. K. Hirst is particularly welcome in this respect. These chapters make the book much more useful as a text for the classroom as

well as serving to introduce the general reader to the basic life-processes of viruses, the most elementary of all living things.

The arrangement of the book represents a trend away from classification by disease and towards a classification by the nature of the causative agent. Infections due to B-virus, the simian member of the herpes group, are now discussed with herpes simplex infections instead of with viral encephalitides. The chapters on dengue and yellow fever are placed near those on the other anthropod-borne diseases. This arrangement facilitates discussion of epidemiology and etiology and detracts little from the book's usefulness for clinical reference.

The new chapters on the ECHO and adenoviruses will doubtless prove useful to anyone concerned with infectious diseases. These newly recognized viruses are responsible for a variety of illnesses which usually are of a minor nature. The extensive diagnostic criteria which are now available are not sufficiently refined to allow identification of most individual cases solely on clinical grounds, but these agents frequently occur in epidemic form, often with striking intensity. Under epidemic conditions clinical diagnosis can become much more accurate and laboratory tests more practical. Perhaps the publication of these diagnostic criteria in this generally available form will mark the beginning of the end of that favorite equivocation, "It's a virus infection."

The preface describes the third edition as "an entirely new book." This is true of many chapters, and others represent thorough revisions. However, a few chapters are almost the same as in the first edition with new data only tacked on the end of subsections rather than integrated into the body of the text. The contributors have been chosen "because of their eminence and experience" rather than because of their current familiarity with these fast-moving subjects. In a few cases they seem to have lost contact with the import of recent developments.

Several other books on viruses are now available but this one remains the most comprehensive and best balanced reference in medical virology. It is highly recommended to anyone concerned with this field.

FRANCIS L. BLACK

CLINICAL EPIDEMIOLOGY. By John R. Paul. Chicago, The University of Chicago Press, 1958. 254 pp., 37 figs. $5.00.

This presentation of the subject of clinical epidemiology is appropriately divided into three components which are concerned respectively with the history, principles, and applications of the subject. The material is introduced and discussed by the author in a concise manner and as an orientation towards the goals and activities of the clinical epidemiologist.

Part I provides a brief discussion and analysis of the historical beginnings and developments of epidemiology as a science from ancient Greece to the present. Herein the author develops his definition of epidemiology as " . . . concerned with the circumstances under which diseases occur, where diseases tend to flourish, and where they do not."

Part II contains the main presentation of the philosophy and principles of epidemiology. The relationships between the causative agents, host sus-

ceptibility, and environments—both the macro-environment and the micro-environment—are considered within the author's conceptual framework of the seed, the soil, and the climate. In addition, the methods and techniques of the experimental and clinical epidemiologist are adequately presented and compared.

Part III is concerned with some of the accomplishments and future plans of the science of epidemiology. This is considered within the context of five separate diseases or disease groupings: rheumatic fever, coronary occlusion, poliomyelitis, infectious hepatitis and serum hepatitis, and the arthropod-borne virus infections. Both previous and proposed applications of epidemiology are evaluated with respect to these categories.

Dr. Paul states in his introduction that this volume was intended to introduce the subject of epidemiology " . . . to doctors and students of medicine, biology, or sociology in non-technical language and with examples they might use." It is the opinion of this reviewer that the author has succeeded in achieving his intent. The presentation of the material is both disciplined and organized. This volume in companionship with *The Uses of Epidemiology* by Jeremy Morris, should provide the student of the medical or behavioral sciences with an excellent foundation for the further study of epidemiology.

WILLIAM L. KISSICK

POLIOMYELITIS. Papers and Discussions Presented at the Fourth International Poliomyelitis Conference. Philadelphia, J. B. Lippincott Co., 1958. 684 pp. $7.50.

The editors of this volume, describing the Fourth International Poliomyelitis Conference held in Geneva in 1957, may well have been hard put to it to maintain the standard set by the three previous volumes in this series, but they have succeeded and are to be congratulated. They have succeeded in issuing another authoritative progress report on poliomyelitis, this time covering the three-year period 1955-1957. The record naturally reflects those features which commanded most attention in North America and Europe at that time. These were the early years of the Salk vaccine, and first on the list are contemporary experiences with this new prophylactic agent. Of particular interest to the reviewer are the experiences from countries outside the U. S. which record the use of this vaccine and their estimates of its value. Included are data which many countries have collected for the first time in an effort to measure that fraction of their population which is susceptible to poliomyelitis. These effects are not limited to the time-honored method of merely recording annually, and on a seasonal basis, the numbers of local cases of poliomyelitis and their geographical location, but many have added quantitative measurements in the form of careful analyses of age-specific antibody surveys. These measurements reflect the global picture of this disease. They also reflect how quickly laboratories all over the world have now taken up the study of polioviruses and its application to estimates of their immediate needs in terms of a program of immunization against poliomyelitis.

Similarly, new proposals relative to vaccination with modified *live* viruses, such as those which have been devised by Koprowski and Sabin, although still in the research phase, receive a good review of the situation up to that date. A third new subject, which has actually only come into being in the last four or five years, is found in the papers dealing with the whole new family of *enteric viruses*—a family to which polioviruses have now been assigned. New relationships have been discovered between the behaviour of these viruses, and the infections they cause, and the behaviour of polioviruses. From the clinical standpoint there is also an excellent critique of newer diagnostic tests—neutralization, complement fixing, and precipitin tests. When one realizes that four years ago laboratory tests for the clinical diagnosis of poliomyelitis on the basis of the immunological response by the patient were rarely performed, the record of the present widespread use of these tests in the diagnostic laboratory is indeed impressive. Also, on the clinical and therapeutic side problems of respiratory distress in patients with life-threatening poliomyelitis receives detailed attention.

No one who wishes to remain abreast of the rapidly moving field of poliomyelitis can afford to be without this book, and, if he is historically minded, he should also try to secure some of the previous volumes in this series.

JOHN R. PAUL

ENVIRONMENTAL INFLUENCES ON PRENATAL DEVELOPMENT. Beatrice Mintz, Ed. Chicago, University of Chicago Press, 1958. 86 pp. $3.00.

This small volume of 86 pages is one of ten reports from The Developmental Biology Conference Series held in 1956 under the auspices of the National Academy of Sciences and the National Research Council. The conferences were held in various research and educational centers in North America under the organization and general chairmanship of Dr. Paul Weiss of the Rockefeller Institute.

Investigators in many fields of biology from various parts of the world presented the results of their researches focused upon the special field for which the conference was held. Working with a conference chairman in each case was an official reporter and editor who was responsible for editing the special volume covering the meeting.

Dr. Beatrice Mintz, assistant professor of biological sciences, University of Chicago and the editor of *Environmental Influences on Prenatal Development*, has brought together in eight chapters the discussions of the panel of 20 who participated in the conference held August 2-4, 1956 at the Roscoe B. Jackson Memorial Laboratory in Bar Harbor, Maine.

The discussions centered around a wide variety of teratological effects produced in embryos and fetuses of many types of animals by different agents involving, for example, x-rays, chemicals, dyes, dietary, endocrine and vitamin deficiencies, physical factors, diseases, and certain inherited tendencies for abnormal development. Many agents were introduced at

different periods in development, and a multiplicity of effects were observed. Different effects could be produced by the same agent at different times in development.

This is quite understandable, for the growing embryo is rapidly changing not only as a whole but at various speeds in many areas. In a long chain of events some cells appear to be more susceptible to certain agents at one time than at another. Damage by an agent introduced at a critical moment in the development of a particular part of an organism may create a disharmony in form that might be totally lacking if applied at another time. This seems to be exemplified in skeletal defects associated with nutritional factors.

It is obvious that different cells at different ages have heavy nutrient requirements for their enzyme systems, and that when the proper inhibiting chemical elements are present, particular defects occur.

There are numerous examples of organs in early development which influence neighboring cells to organize into specific structures. It seems clear that teratogenic agents affecting organs in this critical period set up events which change or inhibit the appearance of the secondary structures that may normally be arising at certain periods in the age of an embryo.

There are many interesting observations recorded in this small volume, but being assembled as a panel discussion of so many types of experiments, it is not as easy reading as in the case of other volumes of this series where the entire manuscripts of the speakers are published along with a summary discussion. The book includes 26 figures and six pages of references.

L. S. STONE

DISEASE, LIFE AND MAN. Essays by Rudolf Virchow. Translated by L. J. Rather. Stanford University Press, 1958. 273 pp. $5.00.

Rudolf Virchow is perhaps best remembered as the founder of cellular pathology. His influence on the rapidly developing medical sciences in the latter half of the 19th century cannot be overestimated; aside from his medical interests, Virchow also achieved prominence in such diverse fields as anthropology and politics.

This book consists of ten essays written at various times in his life, presenting Virchow's unifying philosophy of life and medicine. The essays cover such topics as the conflict between therapeutics and research, cellular pathology as a unifying scientific force in medicine, the conflict between religion and science, and the purpose of the practice of medicine.

A list of the essays included is as follows:

Standpoints in Scientific Medicine (1847)
Scientific Method and Therapeutic Standpoints (1849)
On Man (1849)
Cellular Pathology (1855)
On the Mechanistic Interpretation of Life (1858)
Atoms and Individuals (1859)

Standpoints in Scientific Medicine (1877)
The Place of Pathology among the Biological Sciences (1893)
One Hundred Years of General Pathology (1895)
Recent Progress in Science and its Influence on Medicine and Surgery (1898)

The basis of any science must be experience and observation, religious beliefs of what should be must be put aside in scientific investigation: "The investigator knows only material bodies and properties of bodies. Whatever is beyond he calls transcendent, and he regards transcendence as an aberration of the human spirit."[1]

The idea of knowing only what one's senses can experience permeates all of Virchow's writing and is directly connected to his idea of the cell as the experiential basis of life and disease: "Life is cell activity; its uniqueness is the uniqueness of the cell."[2]

Virchow's idea of the cell as a basic unit was a determining factor in unifying the conflicting schools of pathology of the time, and it was responsible for refounding pathology on a modern, scientific basis. His goals in this matter are stated as follows: "To be sure I have combated the humoral pathology of recent years . . ., but rather to unite both humoral pathology and solidary pathology in an empiracally based cellular pathology. This, I confidently hope, will become the pathology of the future."[3]

The great advances in pathology made in the 104 years since these words were written attest to Virchow's success in attaining his goals and place him as the founder of modern pathology.

Virchow points out that there are three stages in the development of medicine as a science: nature-philosophy, natural history, and natural science. Nature-philosophy substituted "logical" thinking and hypotheses for observation; natural history summarized all past developments in medicine and was built on dogmatic theories. Only natural science finally progressed beyond dogmatic acceptance of authority to observation and experiment. This development was necessary for medicine to become established as a rational science, and it must be adhered to in order to protect the scientific bases of medicine.

In his review of pathology as a science in the late 18th and 19th centuries, Virchow discussed, among other things, the new science of bacteriology. He defines a parasite as a separate entity, living within the human body, not arising from it. He further points out that toxins may be responsible for the noxious action of some bacteria as well as the presence and growth of bacteria per se. He discusses "serum therapy" as a new type of "humoral" pathology, and indicates the importance of cells in producing "substances" having curative effects—those that we now know as antibodies.

The future course of medical science is hinted at in his essay on "Recent Progress in Science" (1898). Hereditary aspects of certain diseases are

[1] Scientific method and therapeutic standpoints (1849).
[2] On the mechanistic interpretation of life (1858).
[3] Cellular pathology (1855).

discussed, and he points out that merely because a disease is found to be familial in incidence does not mean it is hereditarily transmitted; leprosy, for instance, was long held to be a hereditary disease, and a law was actually proposed in the Norwegian Parliament forbidding the marriage of lepers for that reason. It was only with the discovery of the leprosy bacillus by Hansen that the true nature of the disease was recognized. The phenomenon of ectopic growth and its causes are discussed, as is surgical transplantation. The importance of elucidating the mode of action of bacteria and its clinical significance is also discussed; indeed, this remains an important field of basic medical research today.

The book contains an interesting introduction on scientific methodology by Professor Rather, and Virchow's essays are certainly stimulating and thought-provoking. This is an interesting and enjoyable book which serves as a fitting tribute to one of the truly great men of medicine.

D. W. B.

HOST-PARASITE RELATIONSHIPS IN LIVING CELLS. McLaughlin Fellowship Program Symposium. Harriet M. Felton, Ed. Springfield, Ill., Charles C Thomas, 1957. 245 pp.

This small volume consists of formal presentations and informal discussions comprising a symposium held at the University of Texas in April 1956 and sponsored by the McLaughlin Fellowship program. Participants included a number of outstanding authorities, each of whom has made important contributions in the area of host-parasite relationships. Materials and methods available for investigation in this field are discussed and evaluated. The role of the electron microscope in the study of both cell and parasite is ably presented and beautifully illustrated. Sections on cytopathology and the competitive aspects of tissue cell and bacterial physiology review the response of mammalian cells in culture to virus infection and attempt to bridge the gap between classical immunology and enzymology. Current concepts of the host-parasite relationships are presented and include discussions of the morphological basis of the immune response and hypersensitivity, immunity in the field of virology, metabolic interrelationships between host and parasite, and the resistance of the parasite.

All in all, this book makes highly absorbing and informative reading for everyone with any interest or curiosity whatsoever concerning the events that occur when parasite meets host.

ROBERT H. GREEN

LIQUID SCINTILLATION COUNTING. Carlos G. Bell, Jr. and F. Newton Hayes, Eds. Proceedings of a Conference held at Northwestern University, August 20-22, 1957. New York, Pergamon Press, 1958. 292 pp. $10.00.

Liquid scintillation counting is a most powerful and versatile method of radioassay, in particular of soft emitters such as tritium and carbon-14. Unfortunately, the technique is not always a simple one, and indeed some

of its complexities may seem so formidable as to discourage the uninitiated. Even for relatively experienced people in this field, it is not always easy to be fully informed on the current state of the art.

The present volume should therefore be widely welcome. It constitutes the Proceedings of a Conference on Liquid Scintillation Counting held at Northwestern University in August 1957. As such it is an extremely well-edited and organized volume. All major aspects of the field are covered in a manner that is generally concise and understandable and by no means superficial. The individual articles are on a high level of scientific and literary competence. Though short, they provide good introductions and coverage to the various topics. Adequate references to further literature are provided. The arrangement is such that, even though no index is included, the volume is easy to use as a general source book.

Parts I and II are concerned with the scintillation event itself and with the instrumentation necessary to detect it. Having covered these fundamentals, Part III, "The Chemistry of the Counting Sample," deals with the chief problem and limitation of the technique: making compatible and efficient combinations of sample and scintillator. (Actually this topic is not confined to Part III but, as is warranted by its importance, appears again throughout the rest of the book.) Part IV covers the general scope of the technique, from particle physics to archeology and to biology and medicine, and Part V gives in detail some specific applications, including radioassay of biochemical samples. Finally, there are "Developments in Foreign Laboratories" which for some reason are segregated in a separate Part VI.

There seem to be no major omissions in this coverage, although in the opinion of the reviewer, a chapter on the relative advantages and limitations for various applications of liquid scintillation counting, as compared to other methods of radioassay, would have been very useful. The book will be of great value to all whose contact with liquid scintillation counting must extend beyond manufacturers' literature.

RICHARD L. WOLFGANG

THE CENTRAL NERVOUS SYSTEM AND BEHAVIOR. Transactions of the First Conference, February 23-26, 1958. Mary A. B. Brazier, Ed. New York, Josiah Macy, Jr. Foundation, 1959. 450 pp. $5.25.

An edited compilation of statements made by 26 participants in a three-day conference on the contributions by Soviet scientists (and others) to the general problem of the relations between the behavior of organisms and the activities of the central nervous system. The first half of the volume is historical, presenting the views of participants on the works of Russian scientists of the 18th, 19th, and first quarter of the 20th centuries. The first third is an extremely interesting pictorial survey of Sechenov, Danilevsky, Tarkhanov, Wedensky, Ukhtomsky, Pavlov, and Bechterev. This is followed by chapters on their most important works.

The historical perspective here presented provides background for current research in neurophysiology and psychology. This reviewer is not competent to judge the adequacy or the accuracy of this background. It may well be the best that is now available in English. Soviet psychologists are at work on the history of Russian psychology. S. L. Rubenstein has published a volume on the life and works of Sechenov, and B. G. Ananiev is working on a book on Russian psychology of the 18th and 19th centuries.

The second half of the volume is devoted to discussions of post-Pavlovian work on conditioning, to current research on the relation between behavior and brain stimulation, and to electrical correlations of conditioning. The participants in these discussions reported parts of their own research, often in some detail.

This volume does not purport to be a systematic presentation of the research on the topics that were discussed. It is a mixture of factual data, antidotal observations, philosophical comments, with some references to political matters. For example, in his report on Pavlov, Horsley Gantt draws heavily on his unpublished notes taken during the years that he spent in Russia. He reports observations and events which otherwise might never have been published. On page 183 he refers Pavlov's uncompromising adherence not only to scientific principles but also to principles of right and truth in all affairs of life including political.

Although this type of publication may be of little use to research workers, yet its informality contributes to its readability for those who have a general interest in the topics discussed.

MARK A. MAY

PHYSIOLOGY OF FUNGI. By Vincent W. Cochrane. New York, John Wiley & Sons, 1958. xii, 524 pp. $9.75.

Dr. Cochrane has written an up-to-date and eminently readable account of the physiological activities and reactions of the fungi. In his treatise the taxonomic groups of the fungi are not dealt with separately, rather the physiological functions are discussed as separate topics, and examples and demonstrations are drawn from all types of fungi. Thus, we find chapters on cultivation and growth, on chemical composition of fungus cells, on nutrition, metabolism, and reproduction. The book assumes a basic, general biochemical knowledge, but the author does give a certain amount of background information in the introduction of each topic. The book appears to be aimed at graduate students and research workers, and the material is critically discussed at that level. The author has drawn on an impressive volume of literature which also serves as a useful bibliography in following up any particular topic.

The reviewer regrets that the yeasts have been largely excluded from consideration by Dr. Cochrane, although they are admittedly an important

group of fungi. On the other hand, actinomycetes have been included, although they are true bacteria and entirely unrelated to the fungi. The author's reason for including them, namely, that they occupy a similar ecological niche, does not appear compelling. These personal criticisms do not, of course, in any way detract from the value of the book. *Physiology of Fungi* can be recommended to all microbiologists and botanists who are interested in fungi. The volume is attractively printed and bound and remarkably free of misprints.

WOLF VISHNIAC

SURGERY IN WORLD WAR II. (John Boyd Coates, Jr., Editor-in-Chief.) NEUROSURGERY, Vol. I. The Historical Unit, United States Army Medical Service. Washington, D. C., U. S. Government Printing Office, 1958. 465 pp. $5.00.

At the outbreak of World War II the United States Army had no neurosurgical service. In the entire nation there were less than 200 men certified by the American Board of Neurological Surgery, of whom only a portion were available for Army service. The remarkable story of how, within a few short years, a specialty service was built up which capably handled neurosurgical casualties numbering in the tens of thousands is the subject matter of this history. The present volume is divided into a section on administrative considerations and a clinical section on the management of head injuries. A second volume is to deal with injuries of the spinal cord and peripheral nerves.

The administrative portion consists of chapters dealing with the Zone of the Interior and the Mediterranean and European Theaters of Operations. The various other overseas areas receive no mention, probably because the problems were generally the same in all. Indeed, the portions devoted to the Mediterranean are weak and duplicate in general the material given for the European Theater. Subjects covered include the organization of neurosurgical centers, the methods used to train young general surgeons in neurosurgery, the assignment of neurosurgical personnel, the organization of neurosurgical facilities in forward and base areas, as well as the chain of evacuation—all designed to provide the patient with a continuous plan of treatment which might span many months and thousands of miles.

The clinical section on head injuries composes the bulk of the volume. Preserving the vast clinical experience accumulated during the war is the strongest reason for publishing a history of this sort. There are three chapters devoted to the geographical areas mentioned above, which describe in detail the methods of dealing with cranial wounds and their complications in the setting of the military situation. The remaining nine chapters are each an excellent monograph on a specific subject. These include acute craniocerebral injuries due to missiles, wounds of the ventricles, infections, blast injuries, cranioplasty, post-traumatic epilepsy, speech disorders, and clinicopathological aspects of fatal injuries.

The distinguished authors who have contributed to this volume have done an excellent job. The illustrations are numerous and of high quality. My only adverse criticism is directed to the editors, who have allowed a certain amount of repetition of material, but considering the many difficulties involved in assembling a volume of this sort they have succeeded in producing a book which is not only valuable but outstandingly readable.

SAUL A. FRANKEL

ADDITIONAL BOOKS RECEIVED

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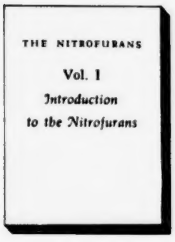

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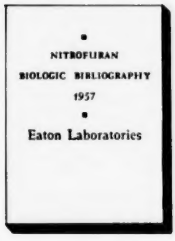

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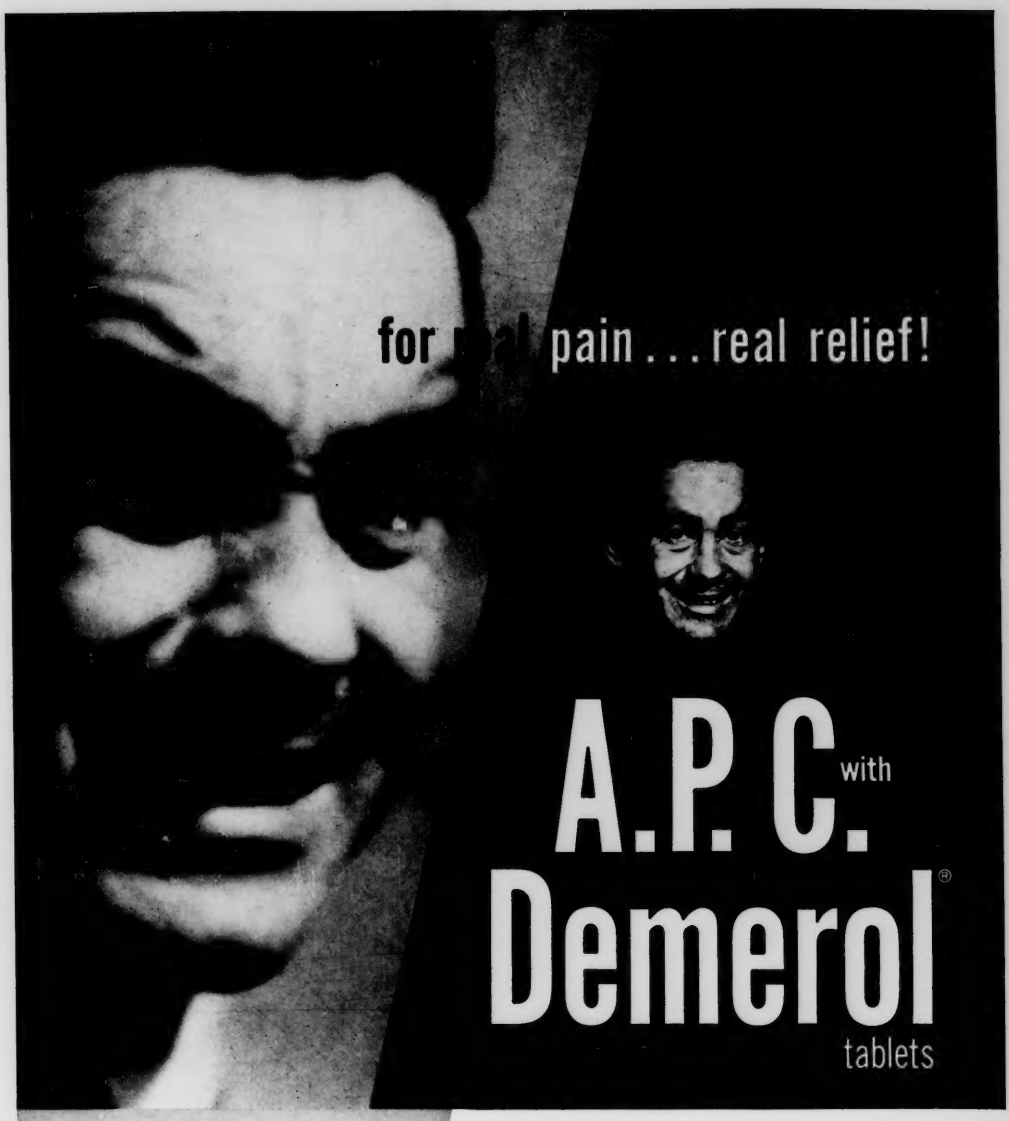

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